IN THE UNITED STATES PATENTS AND TRADEMARK OFFICE

Applicant:	**The Heart Research Institute Ltd. and The University of Queensland**
Serial No:	**10/069923**
Filed:	**4 September 2000**
Title:	**Iron chelators and uses thereof**
Inventors:	**Des Richardson *et al.***

DECLARATION UNDER 37 CFR 1.132

I, Des Richardson of 575 Wainewright Ave, West Hoxton, New South Wales 2171, Australia, do solemnly and sincerely declare as follows:

1. I am a co-inventor of the subject matter of US Patent Application Serial No. 10/069923 (the present application) filed on 4 September, 2001.

2. My qualifications and technical experience are set out in my curriculum vitae, a copy of which is attached as Annexure A.

3. The present application relates to 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH) analogues suitable for use as *in vivo* iron chelators, pharmaceutical compositions containing the analogues and uses of the analogues in the treatment of iron overload.

4. I have read and am familiar with the Office Action mailed 24 September 2004 in respect of the above-referenced application. I understand that the Examiner contends that although the specification does disclose pharmacological data on the chelation activity of PCIH analogues in *in vitro* experiments, the specification does not give any guidance as to whether or not this *in vitro* data correlates to *in vivo* settings, particularly in terms of treating the diseases as claimed.

5. At pages 23-26, the specification describes the effects of iron chelators on iron release from pre-labelled cells and iron uptake from transferrin. A range of experiments were conducted to investigate the efficacy of PCIH analogues PCTH, PCBH, PCBBH, PCAH, PCIH and PCHH compared to "standard chelators" (DFO, PIH and 311). According to our studies, we found that three of these chelators,

namely PCBH, PCBBH and PCTH showed Fe chelation activity that was greater than DFO and comparable to that of PIH and 311 (see page 31, lines 20-22).

6. With regard to the correlation of the *in vitro* data provided in the specification to *in vivo* settings, I attach as Annexure B a journal article for which I am an author, and which is currently in press, entitled "PCTH: A novel orally active chelator of the aroylhydrazone class that induces iron excretion from mice". The in-press article describes my recent investigations of the *in vitro* and *in vivo* effects of PCIH iron chelators, including the following:

(i) Effect of chelators on ^{59}Fe efflux from prelabelled cardiomyocytes

Cardiac complications caused by Fe deposition are a major cause of death in ϑ-thalassemia major patients. Accordingly, to assess the effects of PCTH analogues at mobilizing ^{59}Fe from cardiomyocytes, we pre-labelled cardiomyocytes with ^{59}Fe-Tf (0.75 microM) for 18 h at 37°C, washed and then reincubated them for 24 h at 37°C in the presence of DFO or the one of four most effective PCIH analogues identified from our previous studies (i.e., PCIH, PCTH, PCBH and PCBBH). Three of the PCIH analogues, namely PCTH, PCBH and PCBBH showed significantly ($p<0.005$) greater activity at increasing ^{59}Fe release from pre-labelled cardiomyocytes than DFO. The experiments confirmed the higher Fe chelation activity of PCTH, PCBH and PCBBH compared to DFO that was previously demonstrated using SK-N-MC cells. We found that the chelator with greatest Fe chelation efficacy was PCTH.

(ii) *In vivo* mice studies - acute and subchronic administration of PCTH

The marked activity of PCTH *in vitro* led to studies *in vivo* in mice. Male 6 week old Balb/cmice were used in all studies. For acute studies, the mice were injected with ^{59}Fe bound to lactoferrin at 6 μCi/mg. We used ^{59}Fe-lactoferrin as it has been described to result in hepatocyte ^{59}Fe-labelling in mice (Porter *et al.*, (1990), Blood 76, 2389-2396). Twenty-eight hours after the injection, mice were given by gavage either the vehicle control or a chelator dissolved in the vehicle at the following doses: PCTH (50-200 mg.kg), pyridoxal isonicotinoyl hydrazone (PIH) (200 mg/kg), or deferiprone (L1) (200 mg/kg). The treatments were given twice daily (*bd*), 6 h apart for 2 days, after which the mice were sacrificed. Over three experiments, administration of PCTH induced a significant increase in ^{59}Fe excretion that continued

up to 20 hours after the administration of two doses of treatment, after which it returned to baseline level. The animals remained healthy and no significant weight change was observed even after a high dose of 200 mg/kg/*bd* of PCTH, PIH or L1 was administered over 2 days. Accordingly, we showed that PCTH was orally active and well tolerated by mice in the acute studies.

In subchronic studies, mice were labelled with ^{59}Fe-lactoferrin as above and then 28 h after the initial ^{59}Fe injection, the mice were given by gavage either the vehicle control or PIH or PCTH at a dose of 75 mg/kg/*bd* dissolved in the vehicle. The vehicle was administered for 5 days/week for 3 weeks and faeces collected every 24 h. The ^{59}Fe excreted was calculated per gram of faeces produced. We found that similarly to the acute studies, PIH and PCTH at 75 mg/kg/*bd* increased faecal ^{59}Fe excretion to 140% and 145% of the vehicle control, respectively. In addition there was no change in the weight gain of mice after administration of the chelator for 3 weeks compared to that found for vehicle control. These studies demonstrated that PCTH was orally effective and that Fe chelation efficacy is comparable to the orally active chelators PIH and L1.

(iii) Toxicology

Considering the importance of determining toxicology in the early assessment of drugs for the treatment of human disease, we embarked upon studies to determine the effects of PCTH. Initially, toxicological assessment was examined by comparing oral PCTH administration (50, 100 and 200 mg/kg/*bd*) to the vehicle control over twenty one days by examining mouse body weight and food intake. Mice from all experimental groups except the high dose group of 200 mg/kg/*bd* exhibited normal weight gains over the twenty one day period. Both the 50 and 100 mg/kg/*bd* groups gained a mean of 2% of their initial weight and the increments were not significantly different to the weight gain of the control group of 3%. Food intake by the mice was not significantly different across the treatment groups with the exception of the 200 mg/kg/*bd* group, which consumed 40% less during the first week of treatment when compared to the control.

7. In summary, we found that PCTH demonstrated good oral efficacy and a rapid mode of action in terms of inducing Fe excretion from mice. The effect exerted by

PCTH was equivalent to the known orally active ligands, PIH and L1, indicating its potential as a substitute for DFO. Moreover, the chelator was shown to be well tolerated at doses of 100 mg/kg/*bd* over three weeks. The oral effectiveness of PCTH was further substantiated by native PAGE ^{59}Fe autoradiography studies which demonstrated a reduction in liver ferritin ^{59}Fe in mice administered PCTH. This was in accordance with our data *in vitro* showing that PCTH was capable of inducing ^{59}Fe release from cardiomyocytes and also a number of other cell types. PCTH inhibited ^{59}Fe uptake from ^{59}Fe-Tf into the ferritin of cardiomyocytes which is again in accordance with previous studies using several cell lines. These promising results show good correlation between our *in vitro* and *in vivo* data and support the investigation of these PCIH analogues *in vivo* as prospective therapeutic agents for patients suffering from iron over-load including patients suffering diseases such as β-thalassemia major and Friedreich's ataxia.

8. Turning to the Examiner's assertion that Reference U discloses that 2-pyridylcarboxaldehyde isonicotinoyl hydrazone analogues do not have beneficial effects on Friedreich's ataxia (FA) patients, it appears to me that Reference U is in fact from the inventor's laboratory, namely Chaston T. B. and Richardson D. R., American Journal of Hematology 73: 200-210 (2003). On page 203 of that reference, it refers to PCIH analogues and discloses:

> These tridentate ligands bind Fe(III) [99] and have shown similar chelation efficacy to PIH and low toxicity *in vitro* [98]. Importantly, this class of chelators can access mitochondrial Fe pools, offering a potential treatment for FA [7]. Further *in vivo* studies are under way to determine their efficacy.

Accordingly, in my view, Reference U clearly supports the assertion that PCIH analogues (as described above) have potentially beneficial effects for FA patents. Our further studies *in vivo* indeed support this fact.

9. Finally, Reference V, Abstract from "Richardson's" publication in "Expert Opinion on Investigational Drugs" (2003) 12(2), 235-245 discusses the potential of iron chelators, namely the PCIH analogues as agents to remove mitochondrial iron deposits. These PCIH iron chelators have been specifically designed to enter and

target mitochondrial iron pools, which is a property lacking in desferrioxamine (DFO), the only chelator in widespread clinical use. It is disclosed that standard chelation regimens (i.e., DFO) may not work in FA patients as drugs such as DFO are hydrophilic and cannot cross the mitochondrial membrane. By contrast, the PCIH iron chelators of the present invention preferably comprise a hydrophobic heterocyclic or aromatic group. As disclosed at page 34, lines 14-24 of the present application, our studies identified certain PCIH classes of chelators as highly effective ligands for mobilizing mitochondrial non-heme Fe from reticulocytes. In particular, we identified the ability of PCIH and PCTH to cross the mitochondrial membrane and mobilize mitochondrial Fe pools, thereby overcoming the disadvantage of DFO which cannot effectively deplete Fe from this compartment. These studies further complement the work demonstrating the high chelation efficacy and low toxicity of the PCIH group of ligands in the SK-N-MC neuroepithelioma cell line. Indeed, some of these compounds were far more efficient than DFO at both increasing Fe mobilization from cells and preventing Fe uptake from Tf.

10. In summary, our studies have identified PCIH analogues having desired iron chelating properties both *in vitro* and *in vivo*. In light of the results of our studies, as a person skilled in the art of iron metabolism and iron chelation, I would conclude that the identified PCIH analogues which are described and claimed in US Patent Application Serial No. 10/069923 provide prospective therapeutic agents for Fe-loading diseases such as β-thalassaemia major and FA.

DECLARED at Children's Cancer Institute Australia, Sydney this day of 11/12/ 2004.

Des Richardson

Des Richardson

IN THE UNITED STATES PATENTS AND TRADEMARK OFFICE

Applicant:	**The Heart Research Institute Ltd. and The University of Queensland**
Serial No:	**10/069923**
Filed:	**4 September 2000**
Title:	**Iron chelators and uses thereof**
Inventors:	**Des Richardson *et al.***

ANNEXURE A

This is Annexure A referred to in the Statutory Declaration of Des Richardson made before me this fifteenth day of December 2004.

_____

Curriculum Vitae

Professor Des R. Richardson ***B.Sc., M.Sc., Ph.D, D.Sc.***

NHMRC Principal Research Fellow, Professor, University of New South Wales, Program Head, Childrens Cancer Institute Australia.

TABLE OF CONTENTS

Curriculum Vitae

Section A

1. NAME AND CONTACT DETAILS:

Professor D.R. Richardson, Program Head, Iron Metabolism and Chelation Program.
Children's Cancer Institute Australia, Sydney, New South Wales, 2050
Ph: (02) 9550-3560
FAX: (02) 9550-3302
Email: D.Richardson@ccia.org.au

2. CITIZENSHIP, DATE OF BIRTH, GENDER:

Citizenship: Australian Citizen
Date of Birth: 21/3/1963
Gender: Male
Personal: Married with three children (4, 6 and 10 years old).

3. ACADEMIC QUALIFICATIONS

1983	B.Sc.	University of Western Australia
1984	M.Sc. Preliminary	University of Western Australia
1985	M.B.B.S Yr. 1	University of Western Australia
1987	M.Sc	University of Western Australia
1990	Ph.D	University of Western Australia
2001	D.Sc	University of Western Australia

4. INSTITUTION AND DEPARTMENT

I was both actively recruited and promoted by the Children's Cancer Institute Australia (CCIA). In the CCIA I established my own independent research group of 13 members. My group is housed in 2 well equipped laboratories (including my own cell culture facility), my office, as well as 16 desk spaces for students and post-doctoral fellows. Much of the equipment in these labs has been purchased from my own competitively obtained research funding.

5. CURRENT APPOINTMENTS:

- Professor, Faculty of Medicine, University of New South Wales
- Program Head, Iron Metabolism and Chelation Program, CCIA
- NHMRC Principal Research Fellow

Section B

1. AIMS AND OBJECTIVES OF MY RESEARCH LABORATORIES

AIM: To continue to lead and expand my world-class research program examining: **(1)** the molecular and cellular mechanisms of iron metabolism in normal and neoplastic cells, and **(2)** the use of novel iron chelators as therapeutic agents for the treatment of diseases such as cancer. We aim and succeed in doing world-class research that will be used in the development of therapies for the treatment of major diseases.

OBJECTIVES

To continue to expand our knowledge on the iron metabolism of normal and neoplastic cells and the use of iron chelators as a novel class of anti-tumor agents. This is essential in order to understand the role of iron in cellular growth, proliferation, and the pathogenesis of a variety of diseases.

1. To continue to drive the development of novel iron chelators that have been synthesized, screened, and patented by my lab. These chelators have been designed to replace desferrioxamine for the treatment of cancer and iron overload diseases.
3. To continue to investigate the use of iron chelators for the treatment of cancer and other diseases.
4. To continue to publish in top quality international scientific journals.
5. To continue to teach at the undergraduate and post-graduate levels. I believe that one important contribution of a research scientist is to train and motivate others to do research.
6. To continue to expand the broad array of techniques from X-ray crystallography to gene-knockout technology already in use within my laboratories. This is essential in order to stay at the forefront of my research field.
7. To continue to contribute to the general lay and scientific community at the national and international levels.
8. To continue to patent new ideas and technologies that may lead to new therapies.
9. To continue to obtain research funding from national and international research agencies.
10. To continue to increase the funding of my laboratory from industry. Commercial contracts with Metabolic Pharmaceuticals and Unisearch has been established and BIF funding obtained.

My laboratories are both highly productive (84 publication in the last 6 years) and well funded (15 major national and international research grants). Indeed, these are the major strengths of my lab which is only relatively young (established in Australia from the "ground-up" in Brisbane - July 1997), but has already achieved important research findings.

The high productivity of my labs will continue to increase due to the ever-increasing experience of its members and the collaboration and support from staff of the Children's Cancer Research Institute (HRI), Prince of Wales Oncology Research Centre and Department of Chemistry, University of Queensland. Collaboration with other Senior Investigators of the CCIA has led to work using highly relevant models for screening our chelators as anti-proliferative agents (e.g., use of the NOD/SCID mouse leukemia model). Further, the international reputation of my labs are attracting high quality researchers from abroad as both Ph.D students (Mr. Piao Chin) and visiting scientists (Dr. XiMing Yuan from Prof. Ulf Brunks lab, Sweden).

It should be noted that the patenting of the PCIH group of iron chelators as agents to replace DFO for the treatment of iron overload diseases (eg. β-thalassaemia and Friedreich's ataxia) is an important project that has already attracted investment from industrial partners (e.g., Metabolic Pharmaceuticals and Unisearch Ltd) and a successful Biotechnology Inovation Fund (BIF) grant. Funding of the considerable patent costs by the CCIA demonstrates the strong continuing commitment of the institute to the development of my research program. Preclinical investigation of these compounds is set to begin this year by screening of the most promising chelators in animal models. Success at this level will lead in the future to phase I clinical trials.

Another great strength of my research program is the use of a broad range of advanced techniques ranging from X-ray crystallography to gene-knockout technology. The use of these techniques as well as my continuing quest to use new technologies will enable me to continue to be at the cutting edge of my research field. Indeed, the use of these techniques has been a major attraction for staff, students, and collaborators.

2. EXECUTIVE SUMMARY

General: Professor Richardson is a well established independent investigator and NHMRC Principal Research Fellow. His entire career has been devoted to the field of iron metabolism and iron chelation. Indeed, he has an international reputation in this field, and in 2001 he submitted and was awarded his D.Sc degree based upon this work. He is a relatively young scientist (41 years old) and has published in excess of 130 articles.

Publications and International Reputation: Prof. Richardson heads a dynamic research team consisting of 13 individuals including 7 experienced post-doctoral fellows. Moreover he has extensive experience in managing research projects and staff. His laboratory is highly productive and over the last 6 years has produced 86 publications in this area **(48 since receiving his NHMRC Fellowship in 2002).** On 83 of these papers, Dr. Richardson is either first or senior author, and 76 of these have only 1-3 authors, demonstrating these are primary investigations from his own lab. Thus, his publication record represents a personal contribution rather than one built from extensive collaboration. These articles are published in a variety of high impact international journals including *Cell* (impact factor = 27.2), *Lancet* (13.2), *EMBO J.* (10.7), *Blood* (10.2), *Biochim. Biophys. Acta-Reviews in Cancer* (9.3), *Cancer Res.* (8.3), *J. Immunol* (7), *J. Biol. Chem.* (6.7), *Clin. Cancer Res.* (6.0), *Mol. Pharmacol.* (5.5), *Carcinogenesis* (5.4), *BBA–Bioenergetics* (5.5) *Biochemistry* (4.1), *J Biol Inorg Chem* (3.9) etc.

Prof. Richardson is regularly to write Chapters, Editorials, Commentaries and Reviews for high profile journals (including *Cell* and *Lancet*) ***and is a member of the Editorial Boards of 7 International Journals***, including *Redox Report* (Impact Factor = 1.6), *J. Lab. Clin. Med.* (Impact Factor = 2), *Journal of Inorganic Biochemistry* (Impact Factor = 2.3) *BioMetals* (Impact Factor = 2.6), *Expert Opinion in Investigational Drugs* (Impact Factor = 3.1), *International Journal of Biochemistry and Cell Biology* (Impact Factor = 3.6), and the *Biochemical Journal* (Impact Factor = 4.5). Dr. Richardson is also Editor of the Section *Molecules in Focus* for the *International Journal of Biochemistry and Cell Biology*. Prof. Richardson is frequently invited to examine national and international granting agencies, including the NIH. He has considerable experience in the commercial development of scientific advances and holds 4 patents. The investigators have access to excellent facilities at the Children's Cancer Institute Australia including their own purpose-built laboratory.

Grantsmanship: Prof. ***Richardson was appointed in 2002 to the NHMRC Fellowship scheme (Senior Research Fellow-Level B) and was awarded an early promotion in 2004 to Principal Research Fellow due to his progress.*** He has held prestigious national awards throughout his training including an NHMRC Ph.D Scholarship, MRC of Canada Post-Doctoral Fellowship and MRC of Canada Scholarship. This latter award is 5 year career award for new Assistant Professor's similar to the NHMRC Fellowship award. ***At present, Prof. Richardson holds 14 major grants*** (4 NHMRC grants, 2 ARC Large grants, 2 National Heart Foundation grants, a grant from the Muscular Dystrophy Association USA; 2 commercial grants, Leukaemia Foundation NSW grant etc; Section D). He has consistently won major research funding from national and international granting bodies ***in the large majority of cases as Sole Chief Investigator.***

Scientific Community, Teaching and Institutional Contributions: Prof. Richardson and also his lab members are regularly invited to speak and chair sessions at national and international meetings (eg., Invited Key Participant at the International Friedreich's Ataxia Research Conference, NIH, USA, 2003; Conference Chairs at the International Conference of Oral Iron Chelators, 2003, 2004), and has been actively involved in the organization of numerous national and international conferences. He is currently Treasurer of the Society of Free Radical Research Australasia.

Prof. Richardson continues to be actively involved in a variety of teaching activities (see Section C(g)) including being a regular tutor for the last 4 years in the University of Sydney Medical Course. Since 1997, Prof. Richardson has recruited 13 Ph.D students and was or is Principal Supervisor of 12 of these. Eight of these students have obtained, submitted, or about to submit their Ph.D's. ***In fact, in the last 12 months, 6 Ph.D submissions or completions from his lab have been made.*** Dr. Richardson is mentoring his exceptional Ph.D students and post-docs by involving them in the preparation of research grants and their own post-doctoral fellowship applications. They are also encouraged by travel support packages to present their work at national and international conferences. Prof. Richardson has promoted medical research through a variety of mechanisms (see Section C(k)) and has been actively involved in the management, direction and development of institutional policy at the Dept. Medicine (UQ), Heart Research Institute and Children's Cancer Research Institute via regular Group Leader Meetings with the Director and other senior members of the Institute.

Section C - Detailed Curriculum Vitae

(a) ACADEMIC BACKGROUND

Qualifications

Year	Qualification	Institution
1981-1983	B.Sc	University of Western Australia
1984	M.Sc. Prel.	University of Western Australia
1985	MBBS Yr.1 (Medicine)	University of Western Australia
1986-1987	M.Sc	University of Western Australia
1988-1990	Ph.D	University of Western Australia
2001	D.Sc	University of Western Australia

Academic Achievements

(1) ***Undergraduate B.Sc. Courses***

Majors: Pathology and Biochemistry

* Top Student in Pathology Major
* Science Faculty Commendation for Grade A results in all courses in the 2nd year of BSc. degree
* Awarded a competitive Raine Foundation Summer Research Scholarship (Summer 1992-1993)
* An A grade (Distinction) average was obtained over the 15 courses taken (BSc and MBBS courses).

Distinctions were obtained in: Chemistry 100, Physics 135, Biochemistry 135, Physiology 135, Chemistry 224, Anatomy and Human Biology 212, Biochemistry 200, Microbiology 210, Pathology 300.
Credits (B grade) were obtained in all other subjects.

(2) Postgraduate Courses

M.Sc. Preliminary Grading: - ***1st Class Honours***

Scholarships / Fellowships/ Prizes

(1) ***Raine Foundation Vacation Scholarship*** - Competitive Scholarship in the Faculties of Science and Medicine, University of Western Australia, Department of Biochemistry, Supervisor: Dr. P. Priscott, Summer 1982-83).

(2) ***Commonwealth Postgraduate Scholarship of Australia*** (Ph.D Scholarship, Department of Physiology, University of Western Australia; declined in preference for (3)).

(3) ***National Health and Medical Research Council (NH&MRC) Biomedical Postgraduate Scholarship*** - (Ph.D. Scholarship), Department of Physiology, University of Western Australia (1988-1990).

(4) ***Lady Davis Institute for Medical Research Postdoctoral Fellowship***, Lady Davis Institute, Montréal, Canada, 1992-1993, $25,000 p.a. (declined in preference for (5)).

(5) ***Medical Research Council of Canada Postdoctoral Fellowship*** - Department of Physiology, McGill University and the Lady Davis Institute for Medical Research, Sir Mortimer B. Davis - Jewish General Hospital (1992-1994); Stipend: $30,510 per annum; Research Allowance $1,300 per annum).

(6) ***C.R. Roper Research Fellowship***, University of Melbourne, $43,781 per annum (2 year award) and a grant-in-aid of $4,100 per annum (declined in preference for (8)).

(7) ***Medical Research Council of Canada/Cancer Research Society of Canada Scholarship*** (5 year career award $42,015 stipend/year, $40,000 research grant) - declined in preference for (8). **Letter attached see Section C(m) (letter 1).**

(8) ***Medical Research Council of Canada Scholarship*** (5 year career award: 1994-1999, $42,015 stipend/year, $40,000 research grant, TOTAL VALUE: $250,075). **Letter attached see Section C(m) (letter 1).**

(9) ***Research Fellowship and Senior Research Fellowship,*** Department of Medicine, University of Queensland (July 1997-July 2000).

(10) ***1997 ASMR/AMP Queensland Biomedical Postdoctoral Award*** ($2000).

(11) ***Travel Bursary Award*** (1500 FF) to attend the International Symposium on Iron (Saint Malo, France, 16-20 June, 1997).

(12) ***Travel Award*** of $1000 awarded by the Friedreich's Ataxia Support Group of Queensland to present our research work at the International Meeting on Friedreich's Ataxia, Women's and Children's Hospital Lecture Theatre, Adelaide, South Australia, August 30.

(13) ***Young Investigator Travel Award*** ($500 US) to attend the Chelator Workshop, National Institute of Health (NIH), Bethesda, Maryland, September 21-22, 1998.

(14) ***Thalassemia Centre of NSW Travel Award*** (Return Airfare, $1800) to travel to the 10th International Conference on Oral Chelators, Cyprus, 22-26th of March.

(15) ***Friedreich's Ataxia Association of New South Wales Travel Award 2000*** (Return airfare, $3000) to travel to The International Scholars Meeting, Dailin, China, 2000.

(16) ***Friedreich's Ataxia Association of New South Wales Travel Award 2001*** (Return airfare and registration: $3350) to travel to The 6th International Society for Trace Elements in Humans, Quebec City, September 15-20.

(17) ***National Health and Medical Research Council of Australia Senior Research Fellowship (Level B)*** (5 year career award;grant # 189730; 2002-2006; TOTAL VALUE: $525,000)

(18) ***National Health and Medical Research Council of Australia Principal Research Fellowship*** (Promoted Nov. 2003; Career award 2004-2006; grant # 189730; $120,000/yr; TOTAL VALUE: $360,000)

(b) POSTGRADUATE TRAINING

1984: M.Sc. Preliminary Thesis, Department of Physiology, University of W.A. **Supervisor:** Dr. Erica Baker. ***Productivity:*** 2 publications in peer-reviewed journals, 1 chapter, 1 review (Section E, publication # 1,8,72,73).

1986-1987: M.Sc. Thesis, Department of Physiology, U.W.A. **Supervisors:** Dr. Erica Baker and Dr. John Webb: ***Productivity***: 3 publications in peer-reviewed international journals (see Section E, publications 2,3,5).

1988-1990: Ph.D. Thesis, Department of Physiology, U.W.A **Supervisor**: Dr. Erica Baker. ***Productivity:*** 9 publications in peer-reviewed international journals (see Section E, publications # 4,6,7,9,10,11,12,14,15).

1991: NH&MRC Postdoctoral Research Officer, Department of Microbiology, U.W.A, Perth, Australia. ***Productivity:*** 1 publication in a peer reviewed international journal (see Section E, publication # 13). Please note that this was a new field of research for the applicant (Immunology) so productivity was lower than usual.

1992-1994: Medical Research Council of Canada Postdoctoral Fellow, Department of Physiology, McGill University and the Lady Davis Institute for Medical Research, Montréal, Québec, CANADA. ***Productivity:*** 9 publications in peer-reviewed international journals (see Section E, publications # 16-19,21-25).

Major Postgraduate Techniques Learned

(i) Cell and Molecular Biology

Cell Culture (primary cultures of hepatocytes, cardiomyocytes, macrophages and fibroblasts and culture of a wide variety of neoplastic cell lines), the use of radioisotopes (^{59}Fe, ^{125}I, ^{32}P, ^{3}H, ^{67}Ga, ^{64}Cu, ^{65}Zn), labelling of proteins (transferrin, immunoglobulins) and nucleic acids (random prime labelling, end labelling) with isotopes, pulse-chase labelling of cells with a wide range of isotopes, cellular fractionation, electron and fluorescent microscopy, purification of proteins (pressure dialysis, gel chromatography, membrane filtration techniques, polyacrylamide gel electrophoresis (PAGE), SDS-PAGE), purification and use of monoclonal antibodies, the use of nude mice, Western analysis, horizonal and vertical gel electrophoresis, plasmid purification, spinning column techniques (nucleic acids and proteins), transcription reactions, PCR, Northern analysis, Southern analysis, FACS analysis, nuclear run-on assays, gel-retardation assays, autoradiography, development of novel PAGE and autoradiography techniques to detect Fe-binding proteins (see Richardson et al. 1996 *Blood* 87: 3477-3488).

(ii) Chemistry – Organic, Inorganic and Physical

X-ray crystallography, potentiometry (determination of both acid ionization and formation constants), proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), infrared spectroscopy, UV-Vis spectroscopy, Mössbauer spectroscopy, atomic absorption spectroscopy (AAS), electron paramagnetic resonance spectroscopy (epr), organic and inorganic synthesis of compounds, high pressure liquid chromatography (HPLC), gas-liquid chromatography etc

(c) CURRENT APPOINTMENT

- ***Program Head (Staff Investigator)***, Iron Metabolism and Chelation Group, Childrens Cancer Institute Australia (CCIA). Sydney.
- ***Professor***, School of Women and Children's Health, Faculty of Medicine, University of New South Wales, Sydney.

My research group consists of 13 individuals: myself, 8 full-time post-docs, 1 full-time research assistant that is also a part-time Ph.D student, and 4 full-time Ph.D students.

In the CCIA my research group occupies 2 well equipped purpose-built laboratories, meeting room, and office. All of my students and research staff have their own desks and individual lab space. These laboratories were fully equipped with my own competitively obtained research grants. My labs contain an independent state-of-the-art cell culture facility which includes 2 Clyde-Apac biological safety cabinets, 2 Forma CO_2 cell culture incubators, a Nikon phase-contrast microscope, and an Olympus IX50 phase contrast and fluorescence microscope fitted with photomicrography equipment. Apart from the cell culture facilities, my labs are equipped with a IEC GP refrigerated centrifuge, refrigerated microfuge, 2 standard microfuges, reverse osmosis and 18 mho water purification equipment, autoclave, gel dryer, densitometer, Perkin-Elmer PCR machine, a range of vertical and horizontal gel electrophoresis equipment, 4 power pacs, 4- and 3- decimal place analytical and preparative balances, hybridisation oven, shaking water bath, and a Beckman 640 UV-Vis spectrophotometer. In addition, I have an extensive range of glassware and general lab equipment (eg., 2 pentium laptop computers, 1 pentium desktop, 1 Macintosh Powerbook G3, 2 Macintosh desktops, 2 IBM 486 laptop computers, freezer, 2 refrigerators, pH meter, gilson pipettors and multipipettors etc).

Apart from my own equipment, the Childrens Cancer Institute Australia (CCIA) has a wide range of general equipment including ultracentrifuges, β and γ counters, sonicators, autoclaves, plate-readers etc. The CCIA has a well equipped store which provides a wide range of research consumables (eg. cell culture consumables and reagents etc). I also have excellent access to an epr spectrometer at the HRI for ribonucleotide reductase measurements. Access to the X-ray diffractometer at the Dept. Chemistry (Univ. QLD) is readily available through my collaborator Dr. Paul Bernhardt.

I have built my research group up to this level since the beginning of my research appointment in Australia (July 1997). At the same time I have been successful in obtaining a Senior Research Fellowship and major grant support as Sole Chief Investigator from a number of national (5 x NHMRC, 2 x ARC Large, 1 x Kathleen Cuningham Breast Cancer Foundation, 1 x National Heart Foundaiton), international (National Ataxia Foundation USA and Muscular Dystrophy Association USA) and local funding organisations (see Section D) while continuing to publish papers in high impact journals (see Section E). I strongly believe that this firm foundation will allow me to continue to be a highly productive and successful researcher.

(d) PREVIOUS AND CURRENT ACADEMIC APPOINTMENTS

(i) 1991: ***NHMRC Research Officer***, Department of Microbiology, University of Western Australia.

(ii) 1992-1994: ***Medical Research Council of Canada Postdoctoral Fellow***, Lady Davis Institute for Medical Research, Montréal, and Department of Physiology, McGill University, Montréal, Québec, Canada.

(iii) 1994-1996: ***Tenure-Track Assistant Professor of Medicine and Medical Research Council of Canada Scholar***, Faculty of Medicine, McGill University, Montréal, Québec, Canada ($70,000 start up fund and also a salary $18,000 for a Research Assistant for 7 months). ***Project Director***, Lady Davis Institute for Medical Research of the Sir-Mortimer B. Davis Jewish General Hospital, Montréal, Québec, Canada.

(iv) 1996-1997: ****Tenure-Track Lecturer*** (Level B), James Cook University, Townsville, Australia ($18,000 start up fund), July 1996-July 1997.

(v) 1997-1999: ***Research Fellow*** (Level B; July 1997 – December 1998)/***Senior Research Fellow*** (Level C; Jan. 1999-Nov. 1999), Department of Medicine, University of Queensland. Head of the Cancer Cell Iron Metabolism Research Group.

(vi) Nov. 1999-July 2002: ***Group Leader (Staff Investigator; Appointed at Principal Research Fellow Level),*** The Heart Research Institute. Head of the Iron Metabolism and Chelation Group,. **March 2001-July 2002**: ***Associate Professor***, Faculty of Medicine, University of Sydney.

(vii) ***April 2002-present: Professor***, School of Women's and Children's Health, Faculty of Medicine, University of New South Wales, Australia. ***June 2002-present: Program Head,*** Childrens Cancer Institute Australia, Sydney.

Honorary Appointment

(i) 2000: ***Visiting Professor*** at the Second College of Medical Sciences, Tianjin Medical University, People's Republic of China, 16th June 2000.

Please Note: During my appointment as Lecturer (July 96-July 97) at James Cook University (JCU; Townsville) I could not do any research due to the lack of space and research infrastructure. It should also be noted that I had just arrived from Canada and for the first 6 months I was waiting for approval of my research grants and did not have research funds. I resigned from JCU due to the lack of space and infrastructure and my wish to establish my own research group.

(e) COLLABORATIONS

Current Collaborations (over the last 12 months and continuing)

(1) Dr. Erica Baker, Department of Physiology, University of Western Australia. Characterization of the ferric ammonium citrate-stimulated iron uptake mechanism from transferrin in hepatocytes and understanding its relevance to iron-overload disease (see Section E; recent publication # 39)

(2) Professor Prem Ponka, Lady Davis Institute for Medical Research, Montreal, Quebec, CANADA. Development of orally effective iron chelators for the treatment of iron overload disease (see Section E; recent publications: # 33,35,36,38,67).

(3) Professor Christopher R. Chitambar, Division of Hematology-Oncology, Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin, USA. Examination of the ability of 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone and other aroylhydrazones to inhibit the activity of ribonucleotide reductase in tumour cells (see Section E; recent publications: # 71).

(4) Dr. Paul Bernhardt, Department of of Chemistry, University of Queensland, Brisbane. Design, synthesis, and X-ray crystallographic characterization of novel iron chelators for clinical use (see Section E; recent publications # 42,46,48,68,76,90).

(5) Professor Victor Gordeuk, Howard University, Washington DC. The use of aroylhydrazone chelators as anti-malarial agents (manuscript submitted).

(6) Prof. Pamela Russel (Prince of Wales Oncology Research Centre, Prince of Wales Hospital). The role of the metastasis tumor suppressor, Drg-1, as a potential therapeutic target for Fe chelators.

Past Collaborations

(1) Dr. Erica Baker, Department of Physiology, University of Western Australia. The development of iron chelators for the treatment of iron-overload disease and the molecular mechanisms of cellular iron transport in normal and neoplastic cells (see Section E, publications 1-12,14,15,26,29, 39,73,82).

(2) Dr. P. Ponka, Dr. H.M. Schulman, Lady Davis Institute for Medical Research, Montreal, Canada. Identification and screening of novel iron chelators for the treatment of β-thalassemia in reticulocytes and macrophages and the

molecular mechanisms of cellular iron metabolism and transport (see Section E, publications # 1,8,14,16-19,21-25,27,33,35,36,38,67,73,74,82; see Section D, grant #6).

(3) Dr. P. May, Dr. G. Hefter, Dr. B. Clare, Dr. J. Webb, School of Mathematical and Physical Sciences, Murdoch University, Western Australia and Dr. P. Wilairat, Faculty of Sciences, Department of Chemistry, Mahidol University, Thailand. Estimation and calculation of acid ionization constants and formation constants of clinically useful chelators of the pyridoxal isonicotinoyl hydrazone class (see Section E, publications # 3,5).

(4) Dr. T. St.Pierre, Dr. J. Webb, School of Mathematical and Physical Sciences, Murdoch University, Perth, Western Australia. The use of Mössbauer spectroscopy to examine the intracellular intermediates of iron transport in neoplastic cells (see Section E, publications # 12).

(5) Dr. B. Robinson, Ms. K. Cameron, Dr. A. Rose, Dr. K.J. Turner, Department of Microbiology, University of Western Australia. Isolation of alveolar macrophages from broncho-alveolar lavage specimens for IgE-binding studies to CD23 and the mannose receptor (see Section E, publication # 13).

(6) Dr. W. Jefferies, Dr. R. Gabathuler, and Dr. M. Kennard, Biotechnology Laboratory, University of British Columbia, Vancouver, Canada. Development of melanotransferrin-transfected cell lines to test the function of melanotransferrin in iron uptake (see Section E, publication # 21).

(7) Dr. J.T. Edward and Dr. F.L. Chubb, Department of Chemistry, McGill University, Montreal, Canada. Synthesis of novel iron chelators and the calculation of partition coefficients of these compounds (see Section E, publications # 16,19).

(8) Dr. M.L. Vitolo and Dr. J. Webb, School of Mathematical and Physical Sciences, Murdoch University, Perth, Western Australia. Examination of the chemical stability of pyridoxal isonicotinoyl hydrazone, an iron chelator with potential to treat iron overload disease (see Section E, publications # 2,5).

(9) Dr. Jan Kovar and Dr. Karin Kriegerbeckova, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska, Prague, Czech Republic. Establishment of novel cell lines that grow in transferrin-free, low-iron medium (see Section E, publication # 20).

(10) Dr. Daniel Vyoral, Lady Davis Institute for Medical Research, Montreal, Canada. Development of new techniques to characterize intracellular iron-binding intermediates (see Section E, publication # 25).

(11) Dr. Vera Neumannova, Lady Davis Institute for Medical Research, Montreal, Canada. The effect of nitrogen monoxide on intracellular iron metabolism and the mechanisms involved in the stimulation of cellular proliferation of oxidants (see Section E, publication # 18,20,22,34).

(12) Dr. Greg Anderson, Queensland Institute for Medical Research, Brisbane.The role of mitochondrial iron overload in the pathogenesis of the neurodegenerative disease Friedreichs ataxia (co-investigator on present and past grant applications on this subject – see Section D, grants #17,18 and 21).

(13) Dr Mike Davies, The Heart Research Institute. (1) The use of EPR in quantitating the effect of cytotoxic ion chelators on ribonucleotide reductase activity. (2) The use of Fe chelators as agents against atherosclerosis (see Section D, NHMRC).

(14) Professor Roland Stocker and Dr. Shane Thomas, The Heart Research Institute. Examination of iron Metabolism of the human macrophage and the role of the iron-containing molecule indoleamine 2,3-dioxygenase in macrophage metabolism. (see Section E-publication #66).

(15) Professor Roger Dean, The Heart Research Institute. The role of iron in the oxidation of lipoproteins and their effects on the metabolism of the human macrophage (see Section E-publication #58).

(f) LOCAL, NATIONAL, AND INTERNATIONAL PROFILE

(f-1) Research Seminars

(1) Richardson, D.R. (1984) The use of iron chelators of the pyridoxal isonicotinoyl hydrazone class as agents to treat iron overload disease. Department of Physiology, University of Western Australia, Nedlands, Perth, Western Australia, March 21st .

(2) Richardson, D.R. (1984) Biochemical evaluation of the potential of analogues of pyridoxal isonicotinoyl hydrazone in iron chelation therapy. Department of Physiology, University of Western Australia, Nedlands, Perth, Western Australia, October 31st.

(3) Richardson, D.R. (1987) The role of the tumour antigen, p97 (melanotransferrin), in iron uptake by the human melanoma cell. Fremantle Hospital, Department of Nuclear Medicine, Fremantle, Perth, Western Australia, October 21st.

(4) Richardson, D.R. (1987) Iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective agents to treat iron overload disease. Fremantle Hospital, Department of Nuclear Medicine, Perth, Western Australia, November 28th.

(5) Richardson, D.R. (1989) The mechanisms of iron and transferrin uptake by the human malignant melanoma cell. Department of Physiology, University of Western Australia, Nedlands, Perth, August 20th.

(6) Richardson, D.R. (1989) The metabolism of iron in human melanoma cells with special reference to the use of Mossbauer spectroscopy to investigate iron-binding molecules. School of Mathematical and Physical Sciences, Murdoch University, Perth, Western Australia, October 15, 1989.

(7) Richardson, D.R. (1991) The iron metabolism of the human malignant melanoma cell. Department of Microbiology, Queen Elizabeth II Medical Centre, Nedlands, Perth, Western Australia, May 5.

(8) Richardson, D.R. (1993) The ferric ammonium citrate stimulated iron uptake process in melanoma cells is independent of transferrin receptor mediated endocytosis and the surface-bound diferric transferrin reductase. Lady Davis Institute for Medical Research of the Sir Mortimer B. Davis Jewish General Hospital, Montréal, Québec, CANADA, October 5th .

(9) Richardson, D.R. (1998) The mechanisms of iron and transferrin uptake by human neoplastic cells. Role of the tumour antigen p97 (melanotransferrin). Department of Medicine, Royal Brisbane Hospital, University of Queensland, May 21st .

(10) Richardson, D.R (2001) The development of a melanotransferrin knockout mouse and the function of frataxin in cellular iron metabolism. The Heart Research Institute, 145 Missenden Rd, Camperdown, Sydney, NSW, 2050, Australia, March 26th.

(f-2) Invited Presentations
* Financial Support Provided

(1) Richardson, D.R., Baker, E., Vitolo, M.L., Webb, J. and Ponka, P. (1987) Evaluation of analogues of pyridoxal isonicotinoyl hydrazone for iron chelation therapy. The Annual Postgraduate Student Seminar Series hosted by the Western Australian Branch of the Australian Society for Medical Research, Queen Elizabeth II Medical Centre, Nedlands, Perth, Australia, December 2.

(2) Richardson, D.R. (1989) Role of the transferrin receptor and melanotransferrin (p97) in iron uptake by human melanoma cells. The IXth International Conference on Iron Storage and Transport Proteins, Brisbane, Australia, July 10.

(3) **Richardson, D.R.** (1991) The mechanisms of IgE uptake by human macrophages and a B lymphoblastoid cell line (Wil-2wt): A preliminary report. The 21st Annual Scientific Meeting of the Australian Society of Immunology, Perth, Western Australia, 4-6 December.

(4) **Richardson, D.R.** (1994) An iron uptake mechanism in human cells that is activated by oxygen radicals. Lady Davis Institute for Medical Research of the Sir Mortimer B. Davis Jewish General Hospital, Montréal, Québec, CANADA, January 8.

(5) **Richardson, D.R.**, Neumannova, V., Nagy, E. and Ponka, P. (1994) Effect of nitric oxide on the iron metabolism of neoplastic cells in culture. The XXV Congress of the International Society of Hematology, Cancun, Mexico. In: ***La Revista Investigacion Clinica*** Suppl. page 186.

(6) **Richardson, D.R.** (1995) The effect of the heme synthesis inhibitor, succinylacetone, on the intracellular distribution of iron in rabbit reticulocytes. The Fifth Conference of the International Association for the Study of Disorders of Iron Metabolism, Boston Marriott Copley Place, Boston, Massachusetts, April 11-13

(7) **Richardson, D.R.** (1995) A novel iron uptake mechanism mediated by GPI-anchored human melanotransferrin. International Conference on BioIron, Asheville, North Carolina, April 16-21

(8) **Richardson, D.R.**, Kennard, M.L., Gabathuler, R., Ponka, P. and Jefferies, W.A. (1997) Identification of a novel process of iron uptake mediated by human melanotransferrin. The 1997 AMP-Queensland Biomedical Research Awards, Bancroft Centre Auditorium, Royal Brisbane Hospital, Brisbane, Queensland, June 3. *

(9) **Richardson D.R.** (1997) Energy-dependent mechanisms are responsible for the efflux of some iron chelators but not others. International Symposium on Iron in Biology and Medicine, Saint Malo, France, June 16-20. Satellite Meeting on Iron Chelators. (Invitation could not be accepted due to teaching commitments). *

(10) **Richardson, D.R.** (1998) The role of frataxin in mitochondrial iron overload and its significance to the pathogenesis of Friedreich's ataxia. Neuroimmunology Research Group, Department of Medicine, Clinical Sciences Building, Royal Brisbane Hospital, Brisbane, February 2, 1998.

(11) **Richardson, D.R.** (1998) Mitochondrial iron transport and mitochondrial iron overload: The lessons learnt from developing erythroid cells. International Meeting on Friedreich's Ataxia, Women's and Children's Hospital Lecture Theatre, Adelaide, South Australia, August 30. *

(12) Becker, E. and **Richardson, D.R.** (1998) Development of new iron chelators for the treatment of iron-loading diseases: Aroylhydrazone ligands that permeate the mitochondrion. In: Proceedings of the International Meeting on Friedreich's Ataxia, Women's and Children's Hospital Theatre, Adelaide, South Australia, August 30. ***Invited Presentation given by E. Becker***

(13) **Richardson, D.R.** (1998) Pyridoxal isonicotinoyl hydrazone and its analogues. High affinity iron chelating agents with anti-proliferative activity. Drug Metabolism Group, Department of Medicine, University of Queensland, Brisbane, May 18th.

(14) **Richardson, D.R.** (1999) The effect of potent iron chelators on cellular proliferation and the cell cycle. Department of Physiology, University of Western Australia, Nedlands, Perth, August 18th .

(15) **Richardson, D.R.** (1999) Nitrogen monoxide (NO) and glucose: Unexpected links that affect intracellular iron metabolism and iron mobilisation. Conference of the Australian Physiological and Pharmacological Society, Newcastle, New South Wales, Australia, 28th September.

(16) Watts, R.N. and **Richardson, D.R.** (2000) Nitric oxide, glucose, and iron release from cells. Parting is such sweet sorrow. The Australian Society of Medical Research (NSW) Meeting, Veterinary Faculty Conference Centre, University of Sydney, June 5. ***Invited Presentation given by Ph.D student R. Watts.***

(17) Kwok, J. and **Richardson, D.R**. (2001) The potent anti-tumour agents, anthracyclines, affect crucial regulatory molecules involves in iron metabolism. Proceedings of the ASMR (NSW) Scientific Meeting, The Scientia, University of New South Wales, June 4th. ***Invited Presentation given by Ph.D student J. Kwok***

(18) **Richardson, D.R.** (2001) Development of iron chelators for the treatment of Friedreich's Ataxia. The Annual General Meeting of the Friedreich's Ataxia Association of NSW, August 18th 2001.

(19) Chaston, T.C. and **Richardson, D.R.** (2001) Iron chelators as potential agents for the treatment of Friedreich's ataxia: Analysis of free radical generation by the PCIH ligands. Annual General Meeting of The Friedreich's Ataxia Association of NSW, August 18th 2001. ***Invited Presentation given by Ph.D student T. Chaston.***

(20) **Richardson, D.R.**, Mouralian, C., Ponka, P. and Becker E. (2001) Lipophilic chelators that bind mitochondrial iron: Potential agents for the treatment of Friedreich's ataxia. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(21) Kwok, J.C. and **Richardson, D.R.** (2001) The effect of doxorubicin and its analogues on the iron metabolism of neoplastic cells: Doxorubicin decreases total iron-regulatory protein 1 levels. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia. ***Invited Presentation given by Ph.D student J. Kwok***

(21) Becker, E., Greer, J. and **Richardson, D.R.** (2001) Frataxin expression is regulated by erythroid differentiation and protoporphyrin IX: Clues to a role in heme and iron metabolism of mammalian cells. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia. ***Invited Presentation given by Ph.D student E. Becker***

(22) Becker, E., Lovejoy, D., Watts, R. and **Richardson, D.R.** (2001) Iron chelators with multiple roles: The development and evaluation of compounds for the treatment of iron overload disease and cancer. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia. ***Invited Presentation given by Ph.D student E. Becker***

(23) Watts, R.N. and **Richardson, D.R.** (2001) Novel links between the metabolism of glucose and the mobilization of iron from cells by nitric oxide: The role of glutathione and the identification of ferritin as a major intracellular target. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, December 4th 2001. ***Invited Presentation given by Ph.D student R. Watts.***

(24) Lovejoy, D. and **Richardson, D.R.** (2001) Novel "Hybrid" Iron Chelators Derived from Aroylhydrazones and Thiosemicarbazones Demonstrate High Anti-Proliferative Activity that is Selective for Tumor Cells. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, December 2nd 2001. ***Invited Presentation given by Ph.D student D. Lovejoy.***

(25) Chaston, T.B. and **Richardson, D.R.** (2002) The redox activity of the iron complexes of novel chelators for the treatment of iron overload. Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia. ***Invited Presentation given by Ph.D student T. Chaston.***

(26) Le, N.T.V. and **Richardson, D.R.** (2002) Translation of the potent cell cycle inhibitor $p21^{WAF1/CIP1}$ is dependent on intracellular iron levels. Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia. ***Invited Presentation given by Ph.D student N. Le.***

(27) Chaston, T.C. and **Richardson, D.R.** (2001) DNA-binding and redox activity of the PCIH group of chelators: Implications for their use in the treatment of Friedreich's ataxia. Annual General Meeting of The Friedreich's Ataxia Association of NSW, August 10th 2002. ***Invited Presentation given by Ph.D student T. Chaston.***

(28) **Richardson, D.R.** (2001) Iron chelators and their use in the treatment of Friedreich's ataxia: Examination in Mice. Annual General Meeting of the Friedreich's Ataxia Association of NSW, Children's Cancer Institute, Sydney, August 10th 2002.

(29) Kwok, J. and **Richardson, D.R.** (2002) Unexpected effects of anthracyclines on iron regulatory protein 1 and 2: The anthracycline-iron and anthracycline-copper complexes decrease RNA-binding activity. European Iron Club, Porto, Portugal, September 25-28. ***Invited Presentation given by Ph.D student J. Kwok.***

(30) Kwok, J.C. and **Richardson, D.R.** (2002) Anthracyclines inhibit the iron mobilization pathway from ferritin in cardiomyocytes and neoplastic cells: Inhibition of the ferritin mobilization pathway. European Iron Club, Porto, Portugal, September 25-28. ***Invited Presentation given by Ph.D student J. Kwok.***

(31) **Richardson, D.R.** (2002) The role of iron in the growth of cancer cells and the use of iron chelators as potential therapeutic agents. Annual General Meeting of the Children's Cancer Institute Australia for Medical Research, Sydney Children's Hospital, Randwick, Sydney, NSW, November 14th.

(32) Watts, R.N. and **Richardson, D.R.** (2002) The effects of nitric oxide on cellular iron metabolism. In: Proceedings of The Australian Health and Medical Research Congress. Melbourne Convention Centre, November 25-29. ***Invited Presentation given by Ph.D student R. Watts.***

(33) Watts, R.N. and **Richardson, D.R.** (2002) Nitrogen monoxide and carbon monoxide: mirror-image effector molecules that bind iron but have different effects on cellular iron metabolism. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16. ***Invited Presentation given by Ph.D student R. Watts.***

(34) Kwok, J. and **Richardson, D.R**. (2002) Possible role of superoxide in the inhibition of the iron mobilization pathway from ferritin by anthracyclines. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16. ***Invited Presentation given by Ph.D student J. Kwok.***

(35) Chaston, T.B. and **Richardson, D.R.** (2002) Interactions of the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone analogues with iron and DNA: Implications for the treatment of iron overload disease. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16. ***Invited Presentation given by Ph.D student T. Chaston.***

(36) Food, M., Sekyere, E. and **Richardson, D.R.** (2002) The soluble form of melanotransferrin inefficiently donates iron to cells via non-specific internalization and degradation of the protein. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16. ***Invited Presentation given by Ph.D student M. Food.***

(37) Sekyere, E., Food, M.R. and **Richardson, D.R.** (2002) Expression profile of the transferrin homologue, melanotransferrin (tumour antigen p97), and studies on its thermolysin-like metalloproteinase activity. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania. ***Invited Presentation given by Dr.Eric Sekyere.***

(38) Le, N.T.V. and **Richardson, D.R.** (2002) Intracellular iron levels regulate the nuclear translocation of the universal cell cycle inhibitor $p21^{WAF1/CIP1}$. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania.

(39) Chaston T.B., Lovejoy, D., Watts, R.N. and **Richardson, D.R.** (2002) Iron chelators inhibit neoplastic cell proliferation by different mechanisms of action. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania. ***Invited Presentation given by Ph.D student T. Chaston.***

(40) S.X. Liang and **Richardson, D.R.** (2002) Iron chelation increases $p21^{WAF/CIP1}$ mRNA levels in cells with mutant p53: The possible roles of AP2, SP1, and SP3. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania. ***Invited Presentation given by Dr. Simon Liang***

(41) Lovejoy, D.B. and **Richardson, D.R.** (2002) Novel anti-metabolites for tumour therapy: Iron chelators with high anti-proliferative activity. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania. ***Invited Presentation given by Ph.D student D. Lovejoy.***

(42) **Richardson, D.R.** (2003) Iron metabolism in normal and neoplastic cells and the use of iron chelators for the treatment of iron-loading diseases. Presentation to the Visiting Delegation from the University of Ulster, Northern Ireland. Children's Cancer Institute Australia, April 11th.

(43) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines and lysosomal inhibitors induce iron accumulation in the iron storage protein ferritin. Proceedings of the ASMR (NSW) Conference, Sydney, NSW. ***Invited Presentation given by Ph.D student J. Kwok***

(44) Le, N.T.V and **Richardson, D.R.** (2003) Potent iron chelators affect multiple genes involved in the p53 tumour suppressor pathway and cell cycle control: A gene array study. In: Proceedings of the 13th International Conference on Oral Chelation and Therapy, July 13-15th, Prague, Czech Republic. ***Invited Presentation given by Ph.D student N. Le.***

(45) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines and lysosomal inhibitors induce iron accumulation in the iron storage protein ferritin by preventing iron mobilization from the molecule. In Proceedings of the 13th International Conference on Oral Chelation and Therapy, July 10-15th , Prague, Czech Republic.

(46) Yuan, J., Lovejoy, D.B. and **Richardson, D.R.** (2003) Investigations of the anti-tumour effects and apoptotic pathways induced by iron chelators in vitro and in vivo. The 13th International Conference on Oral Chelation and Therapy, July 13-15th, Prague, Czech Republic. ***Invited Presentation given by Post-doctoral Fellow Dr. J. Yuan.***

(47) Wong, C. and **Richardson, D.R**. (2003) The novel iron chelator, PCTH, is orally active and induces iron excretion from mice that is comparable to deferiprone and pyridoxal isonicotinoyl hydrazone. The 13th International Conference on Oral Chelation and Therapy, July 13-15th, Prague, Czech Republic. ***Invited Presentation given by Post-doctoral Fellow Dr. C. Wong.***

(48) Le, N.T.V and **Richardson, D.R.** (2003) Potent iron chelators increase the mRNA levels of the universal cyclin-dependent kinase inhibitor, $p21^{CIP1/WAF1}$, but paradoxically inhibit its translation: A potential mechanism of cell cycle dysregulation. The 13th International Conference on Oral Chelation and Therapy, July 13-15th , Prague, Czech Republic. ***Invited Presentation given by Ph.D student N. Le.***

(49) Liang, S.X. and **Richardson, D.R.** (2003) The effect of iron chelators on p53: Iron chelation increases WAF1 mRNA expression by a p53-independent pathway. The 13th International Conference on Oral Chelation and Therapy, July 13-15th , Prague, Czech Republic.

(50) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines inhibit ferritin iron mobilization: A novel additional mechanism of cytotoxicity. Tow Prize Meeting, Sydney, NSW, Nov. 7. ***Invited Presentation by Post-doc Dr. J. Kwok.***

(51) Kwok, J.C. and **Richardson, D.R.** (2003) National ASMR conference, Adelaide, South Australia, Nov. 23-25th . ***Invited Presentation by post-doc Dr. J.C. Kwok***

(52) Castelnoble, L.A. and **Richardson, D.R.** (2003) The role of N-myc in the regulation of iron metabolism in neuroblastoma cells. National ASMR Conference, Adelaide, South Australia, Nov. 23-25th. ***Invited Presentation by Ph.D student L. Castelnoble.***

(53) Nurtjahja-Tjendraputra, E., Le, N.T.V. and **Richardson, D.R.** (2003) Potent iron chelators affect the expression of multiple molecules involved in cell cycle progression, metastasis, DNA excision, and apoptosis. The Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28. ***Invited Presentation by Post-doc Dr. E. Nurtjahja-Tjendraputra.***

(54) Wong, C.S.M. and **Richardson, D.R.** (2003) The acute and subchronic effect of PCTH on iron excretion in mice. The Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28. ***Invited Presentation by Post-doc Dr. C. Wong***

(55) Le, N.T.V. and **Richardson, D.R.** (2003) The metastasis suppressor gene, *NDRG1*, is markely up-regulated by iron chelators but not the DNA-damaging agent, actinomycin D. The Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28. ***Invited Presentation by Ph.D student N. Le***

(56) Xie, L., Napier, I.A. and **Richardson, D.R.** (2003) Examination of the pathophysiological function of frataxin using cells transfected with an anti-sense frataxin expression vector. The Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28. ***Invited Presentation by Ph.D student I. Napier***

(57) Castelnoble, L.A., Le, N.T.V., and **Richardson, D.R.** (2003) Iron chelators overcome N-MYC repression of NDRG1 in neuroblastoma cells. The Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28. ***Invited Presentation by Ph.D student L. Castelnoble.***

(58) Dunn. L.L., Sekyere, E.O. and **Richardson, D.R.** (2003) The melanoma tumour antigen p97 (Melanotransferrin): Expression studies and splice variants in humans and mice. The Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28. ***Invited Presentation by Ph.D student L Dunn***

(59) **Richardson, D.R.** (2003) The molecular mechanisms involved in the inhibition of the cell cycle and cellular proliferation by potent iron chelators. Prince of Wales Oncology Research Centre, Prince of Wales Hospital, Sydney, Dec. 17th.

(60) **Richardson, D.R**. (2003) Iron chelators and the metabolism of iron in cancer cells. Metals in Medicine Network NSW Meeting, School of Chemistry, University of Sydney, Jan. 19th

(61) Castelnoble, L.A., Le, N.T.V and **Richardson, D.R.** (2004) Iron chelators overcome N-myc repression of Ndrg1 in neuroblastoma cells. European Society for Clinical Investigation and Fourteenth International Conference on Oral Chelation, Utrecht, The Netherlands, April 14-17th. ***Invited Presentation by Ph.D student L. Castelnoble.***

(62) Le, N.T.V. and **Richardson, D.R.** (2004) Potent iron chelators but not the DNA-damaging agent actinomycin D markedly up-regulate the metastasis suppressor gene, Ndrg1. European Society for Clinical Investigation and Fourteenth International Conference on Oral Chelation, Utrecht, The Netherlands, April 14-17th. ***Invited Presentation by Ph.D student N. Le***

(63) Yuan, J., Lovejoy, D.B. and **Richardson, D.R.** (2004) Novel Di-2-Pyridyl-Derived Iron Chelators with Marked and Selective Anti-Tumor Activity: *In Vitro* and *In Vivo* Assessment. European Society for Clinical Investigation and Fourteenth International Conference on Oral Chelation, Utrecht, The Netherlands, April 14-17th.

(64) Watts, R.N. and **Richardson, D.R.** (2004) The Mobilization of an NO-Fe Complex from Cells is Mediated by Active Transport – The Possible Role of the Glutathione Conjugate Transporter, MRP1. European Society for Clinical Investigation and Fourteenth International Conference on Oral Chelation, Utrecht, The Netherlands, April 14-17th.

(65) Richardson, D.R. (2004) The potential of anti-cancer drugs with a novel mechanisms of action: New anti-metabolites. Progen Pharmaceuticals, Brisbane, Queensland, May 10th.*

(66) Napier, I and **Richardson, D.R.** (2004) The function of frataxin in mitochondrial iron metabolism and the Friedreich's ataxia knockout mouse. Friedreich's Ataxia in New South Wales Research Meeting, July 31st, Children's Cancer Institute Australia, Sydney. ***Invited Presentation by Ph.D student Ian Napier***

(67) **Richardson, D.R.** (2004) Overview and update on progress in the Friedreich's ataxia research field. Friedreich's Ataxia in New South Wales Research Meeting, July 31st, Children's Cancer Institute Australia, Sydney.

(68) Park, J and **Richardson, D.R.** (2004) Development of cellular models of Friedreich's ataxia. Friedreich's Ataxia in New South Wales Research Meeting, July 31st, Children's Cancer Institute Australia, Sydney. ***Invited Presentation by Post-doc Dr. Jenny Park.***

(69) Nurtjahja-Tjendraputra, E., Le, N.T.V. and **Richardson, D.R.** (2004) Iron chelators: Novel anti-tumour agents that induce cyclin D degradation and affect the expression of molecules involved in cell cycle progression, DNA excision and apoptosis. Australian Society of Medical Research NSW Conference, Sydney, June 7. ***Invited Presentation by Post-doc Dr. E. Nurtjahja-Tjendraputra***

(70) **Richardson, D.R.** and Le, N.T.V. (2004) Expression of the metastasis and growth suppressor gene, *NDRG1*, is

regulated by intracellular iron via HIF-1α-dependent and -independent mechanisms. European Iron Club Conference, Rennes, France, September 10.

(71) Davies, N., Chitambar, C.R. and **Richardson, D.R.**, (2004) Resistance to gallium nitrate in human leukaemic cells is associated with marked alterations in iron tranfficking pathways European Iron Club Conference, Rennes, France, September 10. ***Invited Presentation by Post-doc Dr. Neil Davies.****

(72) Dunn, L.L. and **Richardson, D.R.** (2004) Inhibition of melanotransferrin (p97) expression in melanoma cells inhibits proliferation and cellular migration. European Iron Club Conference, Rennes, France, September 8. ***Invited Presentation by Ph.D student, Louise Dunn*.***

(73) Park, J. and **Richardson, D.R.** (2004) Effects of frataxin hyper-expression on intracellular iron trafficking. European Iron Club Conference, Rennes, France, September 10. ***Invited Presentation by Post-doc, Dr. J. Park****

(74) **Richardson, D.R.** and Nurtjahja, E. (2004) Iron chelators: Novel anti-tumour agents that inhibit cell cycle progression and induce decreased cyclin D1 and P21 expression. European Iron Club Conference, Rennes, France, September 8-11

(75) **Richardson, D.R.**, Yuan, J. and Lovejoy, D. (2004) Novel DpT Fe chelators show marked and selective anti-tumour activity in vitro and in vivo. European Iron Club Conference, Rennes, France, September 8-11.

(76) **Richardson, D.R.** (2004) Anti-cancer drugs with a novel mechanism of action: new anti-metabolites. Anti-soma Inc., London, UK, September 15

(77) Davies, N., Chitambar, C.R. and **Richardson, D.R.** (2004) Resistance to gallium nitrate in human leukaemia cells is associated with marked alterations in iron trafficking pathways. Second Australian Health and Medical Research Congress, MEPSA Section, November 21-26. ***Invited Presentation by Post-Doc, Dr. N. Davies.***

(78) Whitnall, M., Howard, J. and **Richardson, D.R.** (2004) Novel anti-tumour agents show rapid and broad activity against a range of cancer cells and overcome resistance to clinically used cytotoxics. Second Australian Health and Medical Research Congress, MEPSA Section, November 21-26. ***Invited Presentation by Post-Doc Dr. Jonathan Howard.***

(79) Dunn, L.L. and **Richardson, D.R.** (2004) Inhibition of melanotransferrin (p97) expression in melanoma cells inhibits proliferation and cell migration. Second Australian Health and Medical Research Congress (AHMRC), MEPSA Section, November 21-26. ***Invited Presentation by Ph.D student L. Dunn.***

(80) Le, N. and **Richardson, D.R.** (2004) Unexpected links between iron metabolism and the expression of the metastasis suppressor gene, *Ndrg1*: Iron depletion using chelators up-regulates *Ndrg1* expression. Second Australian Health and Medical Research Congress (AHMRC), MEPSA Section, November 21-26.

(81) Dunn, L.L., Yahmato, Y., Sekyere, E.O. and **Richardson, D.R.** (2004) Elucidating the function of the melanoma tumour antigen p97 (melanotransferrin). Oxidants and Anti-Oxidants in Chemistry and Biology. The Thirteenth Annual Conference of the Society of Free Radical Research Australasia, Christchurch, New Zealand, 3-5 Dec. ***Invited Presentation by Ph.D Student, Y. Yahmato.***

(82) Watts, R.N. and **Richardson, D.R.** (2004) A Radical Transporter and a New Model of NO Release?: The Detoxifying Protein MRP1 Assists in Nitric Oxide (NO)-Mediated Fe Export from Cells. The Thirteenth Annual Conference of the Society of Free Radical Research Australasia, Christchurch, New Zealand, 3-5 Dec.

(83) **Richardson, D.R.** (2004) Development of novel and selective anti-tumor agents: Chelators of the DpT class. Genscreen Commercialisation Workshop, Children's Cancer Institute Australia for Medical Research, Dec 1st.

(f-3) Invited Speaker

*** *Financial Support Provided***

(1) **Richardson, D.R.** (1987) The use of pyridoxal isonicotinoyl hydrazone and analogues as iron chelators for the treatment of iron overload. Annual meeting of the Victorian Thalassemia Society, October 15, Melbourne, Australia.*

(2) **Richardson, D.R.** (1992) The role of the transmembrane oxidoreductase in iron uptake from transferrin. Department of Chemistry and Biochemistry, Concordia University, Montreal, Québec, Canada, August 18.

(3) **Richardson, D.R.** (1993) The mechanisms of iron uptake from transferrin by cells in culture. The 76th Canadian Society for Chemistry Conference and Exhibition, Sherbrooke, Quebec, Canada, May 30-June 3. *

(4) **Richardson, D.R.** (1995) The mechanisms of iron uptake by the human malignant melanoma cell. The Departments of Clinical Biochemistry and Cellular and Molecular Pathology, University of Toronto, Toronto, Canada, June 28.*

(5) **Richardson, D.R.** (1995) Multiple iron uptake pathways in neoplastic cells. Is melanotransferrin (p97) involved in iron uptake by melanoma cells ? The International Chemical Congress of the Pacific Basin Societies, Honolulu, Hawaii, December 17-22. *

(6) **Richardson, D.R.** (1996) Identification of a mechanism of iron uptake by cells that is stimulated by preincubation with small molecular weight iron complexes. Department of Physiology, University of Western Australia, January 12.

(7) **Richardson, D.R.** (1996) Mechanisms involved in the metabolism of iron in normal and neoplastic cells. Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland, March 5, 1996.

*(8) **Richardson, D.R.** (1996) Metabolic pathways involved in the uptake and utilisation of iron by normal and neoplastic cells. The Queensland Institute of Medical Research, The Bancroft Centre, Brisbane, Queensland, November 20. *

(9) **Richardson, D.R.** (1997) The molecular processes involved in the metabolism of iron in melanoma cells and the role of melanotransferrin (p97) in iron uptake. Griffith University, The School of Biomolecular and Biomedical Sciences, Faculty of Science and Technology, February 14, Brisbane, Queensland.*

(10) **Richardson, D.R.** (1997) The molecular mechanisms of iron and transferrin uptake by melanoma and neuroblastoma cells and the use of iron chelators as effective anti-proliferative agents. The Queensland Institute of Medical Research, The Bancroft Centre, Herston, Brisbane, Queensland, April 30. *

(11) **Richardson, D.R.** (1997) Invited by Professor Ioav Cabantchik to take part in a special conference of 40 leaders in the iron metabolism and chelation field, Israel. Unfortunately, I could not attend this conference due to teaching commitments -Financial support offered.

(12) **Richardson, D.R.** (1998) Activation of an iron transport mechanism in hepatocytes by preincubation with low molecular weight iron complexes: Implications for the Pathogenesis of Iron Overload Disease. Asian Pacific Association for the Study of the Liver, 11th Biennial Scientific Meeting, 16-20th February, Burswood International Resort, Perth, Western Australia.

(13) **Richardson, D.R.** (1998) Frataxin: Its possible role in mitochondrial iron metabolism. The Murdoch Institute for Research into Birth Defects, Royal Children's Hospital, Flemington Rd, Parkville, Melbourne, Australia, Feb. 23. *

(14) **Richardson, D.R.** (1998) Membrane iron transport mechanisms in neoplastic cells. The Department of Physiology and Pharmacology, University of Queensland, Brisbane, March 20.

(15) **Richardson, D.R.** (1998) Human malignant melanoma cells: A craving for iron. Department of Biochemistry, University of Adelaide, Adelaide, South Australia, June 15th.

(16) **Richardson, D.R.** (1998) Iron transport mechanisms and intracellular intermediates involved in iron meabolism. National Research Centre for Environmental Toxicology, 39 Kessels Rd, Coopers Plains, Brisbane, Queensland, July 14th.

(17) Richardson, D.R. (1998) Molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cell. Feist-Weiller Centre for Cancer Research, Louisiana State University Medical Center, 1501 Kings Highway, Shreveport, Louisianna, USA, September 23rd. *

(18) Richardson, D.R. (1998) Molecules involved in iron transport and iron release in normal and neoplastic cells – the regulation of the Nramp2 transporter and the role of ceruloplasmin in iron efflux. Lady Davis Institute for Medical Research of the Sir- Mortimer B. Davis Jewish General Hospital, Montreal, Quebec, Canada, September 24th. *

(19) Richardson, D.R. (1998) The mechanisms of iron transport and the use of iron chelators as effective anti-neoplastic agents. Department of Medicine (Division of Hematology/Oncology), Medical College of Wisconsin, Milwaukee, WI, USA, September 25th *

(20) Richardson, D.R. (1999) The use of iron chelators as effective anti-neoplastic agents. Department of Pharmacy, University of Queensland, Brisbane 10th March.

(21) Richardson, D.R. (1999) The role of iron in the development of Friedreichs Ataxia and the use of iron chelators for treatment. Friedreich's Ataxia Support Group of Queensland, East League Club, Brisbane, Queensland, April 8th .

(22) Richardson, D.R. (1999) The effect of a novel iron chelator on molecular targets involved in cellular proliferation: The possible roles of WAF1 and GADD45 in cell cycle arrest. ***Plenary Session 3***, Iron Toxicity from Basic Mechanisms to Therapeutic Intervention. World Congress on Iron Metabolism, Bioiron'99, Sorrento, Italy, May 25.

(23) Richardson, D.R. (1999) The effect of iron chelators and nitrogen monoxide on gene expression, the cell cycle, and intracellular iron metabolism. Institute of Hematology and Blood Transfusion, U nemocnice 1, 128 20, Prague 2, Czech Republic, June 2nd . *

(24) Richardson, D.R. (1999) The role of melanotransferrin, ceruloplasmin and Nramp2 in iron metabolism. Institute of Pathophysiology, Faculty of Medicine, Charles University, Prague, Czech Republic June 3rd .

(25) Richardson, D.R. (1999) Iron transport and iron efflux mechanisms in neoplastic cells: Ceruloplasmin is involved in stimulating iron efflux but not iron uptake. Cell Growth Control Laboratory, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague 142 20, Czech Republic, June 3rd.

(26) Richardson, D.R. (1999) Iron chelators as potent anti-proliferative agents for the treatment of neoplasia: Effects on the cell cycle control molecules GADD45 and WAF1. Queensland Pharmaceutical Research Institute, Mt. Gravatt Research Park, Nathan, Brisbane, Queensland, June 28th.

(27) Richardson, D.R. (1999) The chemistry and biological activity of aroylhydrazone iron chelators: Potential ligands for the treatment of iron overload diseases and cancer. Department of Chemistry, University of Queensland, St. Lucia, Queensland, July 28th .

(28) Richardson, D.R. (1999) The molecular mechanisms of iron uptake and release – the roles of Nramp2, ceruloplasmin and melanotransferrin. Department of Physiology, University of Western Australia, Perth, Nedlands, Western Australia, 18th August.

(29) Richardson, D.R. (1999) Molecular controls of cellular iron metabolism and the cell cycle: Iron transport and the use of iron chelators as potential anti-tumour agents, Westmead Institute for Cancer Research, Westmead Hospital, Westmead, Sydney, NSW, August 23rd.

(30) Richardson, D.R. (1999) The regulation and roles of Nramp2, ceruloplasmin and melanotransferrin in cellular iron metabolism. Heart Research Institute, 145 Missenden Rd, Camperdown, Sydney, NSW 2050, Australia, August 24th . *

(31) Richardson, D.R. (1999) Iron chelation therapy: Structure activity relationships of iron chelators and the effects of these ligands on the expression of genes involved in G_1/S arrest. Heart Research Institute, 145 Missenden Rd, Camperdown, Sydney, NSW 2050, Australia, August 25th. *

(32) Richardson, D.R. (1999) Friedreich's ataxia: Iron accumulation in the mitochondrion and the possible use of iron chelators as therapeutic agents. Friedreichs Ataxia Association of New South Wales, Golden Wing Club, Sydney Airport, Sydney, NSW, August 25th.

(33) Richardson, D.R. (1999) Effect of iron chelators with potent anti-proliferative activity on the expression of molecules involved in cell cycle progression and proliferation. Proceedings of the International Conference on Oxidative Pathways in Health and Disease, Veterinary Faculty Conference Centre, University of Sydney, Sydney, 1st of December.

(34) Richardson, D.R. (2000) New orally effective iron chelators for the treatment of β-thalassemia: The 2-pyridylcarboxaldehyde isonicotinoyl hydrazone analogues. Thalassemia Centre of New South Wales, Queen Mary Hospital, New South Wales, 12th January.

(35) Richardson, D.R. (2000) Multiple effects of iron chelators on molecules controlling cell cycle progression. The 10th International Conference on Oral Chelators, Limassol, Cyprus, 22-26th of March. *

(36) Richardson, D.R. (2000) A relationship between glucose metabolism and NO-mediated iron mobilization from cells. The 10th International Conference on Oral Chelators, Limassol, Cyprus, 22-26th of March. *

(37) Richardson, D.R. (2000) Nitrogen monoxide-mediated iron efflux: The roles of glucose, glutathione, and intracellular redox state. 2nd International Conference on HIV and Iron, March 31st -April 1st , Brugge, Belgium. *

(38) Richardson, D.R. (2000) Iron chelators as potential anti-neoplastic agents: Their effect on molecules involved in proliferation. 6th International Symposium on Metal Ions in Biology and Medicine, "Metals in Oncology Session", 7 - 10th May, San Juan, Puerto Rico, USA.*

(39) Richardson, D.R. (2000) Analogues of pyridoxal isonicotinoyl hydrazone as new orally effective chelators for the treatment of iron overload disease. The New South Wales Thalassemia Group, May 22nd

(40) Richardson, D.R. (2000) Molecules involved in cell cycle progression are markedly affected by iron chelators. Department of Medicinal Chemistry, University of Vienna, Austria, April 2nd .

(41) Richardson, D.R. (2000) The effect of nitrogen monoxide (NO) on intracellular iron metabolism. An intricate link between energy metabolism and iron efflux. Institute of Hematology and Blood Transfusion, U nemocnice 1, 128 20, Prague 2, Czech Republic, April 3rd. *

(42) Richardson, D.R. (2000) The effect of iron and erythroid differentiation on the expression of molecules involved in Fe transport and cell cycle control. Cell Growth Control Laboratory, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague 142 20, Czech Republic, April 3rd.

(43) Richardson, D.R. (2000) Iron chelators as potential anti-neoplastic agents: Their effect on molecules involved in proliferation. 6th International Symposium on Metal Ions in Biology and Medicine, "Metals in Oncology Session", 8th May, Caribe Hilton Hotel, San Juan, Puerto Rico, USA. *

(44) Richardson, D.R. (2000) The anti-proliferative effects of the iron chelator, 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone, and its effects on molecules involved in G_1/S progression and regulation of the cell cycle. Centre for Sickle Cell Disease, Howard University, Washington DC 20059, USA, May 10th .

(45) Richardson, D.R. (2000) The effect of nitrogen monoxide on the RNA-binding activity of iron-regulatory protein 1 (IRP1) and intracellular iron metabolism. Lady Davis Institute for Medical Research of the Sir- Mortimer B. Davis Jewish General Hospital, Montreal, Quebec, Canada, May 11th. *

(45) Richardson, D.R. (2000) The development of novel iron chelators for the treatment of β-thalassemia. The New South Wales Thalassemia Group, Concord Hospital, Sydney, New South Wales, May 22nd.

(46) Richardson, D.R. (2000) Mitochondrial iron overload in the pathogenesis of Friedreich's ataxia and the use of iron chelators as agents to treat this disease. Friedreich's Ataxia Association of New South Wales Meeting, Concord Hospital, Sydney, New South Wales, August 12th.

(47) Richardson, D.R. (2000) Oral iron chelation therapy - novel hydrazones with high iron chelation activity. Australian Thalassemia Association, Central New South Wales Coast, October 28th.*

(48) Richardson, D.R. (2001) Friedreich's ataxia: The role of frataxin in cellular iron metabolism and the use of novel iron chelators that permeate the mitochondrion as therapeutic agents. Friedreichs Ataxia Association of New South Wales Annual General Meeting, The Heart Research Institute, Sydney, NSW, August 18th.

(49) Richardson, D.R. (2001) The development of novel iron chelators as selective anti-tumor agents: Molecular and cellular analysis of the role of iron in proliferation. The James Brown Cancer Centre, Louisville, Kentucky, USA, September 9th . *

(50) Richardson, D.R. (2001) Iron chelators selectively inhibit the proliferation of tumor cells: Molecular and cellular mechanisms of action. Lady Davis Institute for Medical Research of the Sir- Mortimer B. Davis Jewish General Hospital, Montreal, Quebec, Canada, September 19th . *

(51) Richardson, D.R. (2001) Aims and activities of the Heart Research Institute. Rotary Club of Sydney (Darling Harbour Branch), Grace Hotel, Sydney, November 28th.

(52) Richardson, D.R. (2001) Iron and cellular proliferation: The use of potent iron chelators as anti-tumor agents. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney December 2nd.*

(53) Richardson, D.R. (2002) Molecular role of iron in cell cycle progression in neoplastic cells: Development of potent iron chelators as anti-tumor agents. Centenary Institute, Sydney, January 22nd

(56) Richardson, D.R. (2002) Molecular characterisation of potent iron chelators that act as anti-proliferative agents against neuroblastoma and other tumors. The Childrens Cancer Institute Australia, Randwick, Sydney, New South Wales, February 4th

(57) Richardson, D.R. (2002) Molecular roles of iron in cell cycle progression: The use of novel iron chelators as anti-tumour agents. Department of Clinical and Molecular Genetics, Royal Alfred Hospital, Level 6, 28th March.

(58) Richardson, D.R. (2002) Multiple iron transport pathways in neoplastic cells: The roles of transferrin and melanotransferrin (tumour antigen p97) in iron uptake. Minisymposium-Biomolecular Transport, Science Faculty, Charles University, Prague, Czech Republic, April 12th. *Biorad Sponsored Speaker**

(59) Richardson, D.R. (2002) Anthracycline-mediated alterations in iron-regulatory protein-RNA-binding activity: The iron and copper complexes of anthracyclines decrease RNA-binding activity. Institute of Hematology and Blood Transfusion, U nemocnice 1, 128 20, Prague 2, Czech Republic, April 15th. *Biorad Sponsored Speaker* *

(60) Richardson, D.R. (2002) The mechanism of nitric oxide mediated iron release from cells: Relevance to the cytotoxic effector mechanisms of activated macrophages against tumor cells. The Department of Pathology, University of Linkoping, Sweden, April 17th. *

(61) Richardson, D.R. (2002) Molecular effects of anthracyclines on intracellular iron processing and trafficking pathways. Muscle Research Unit, Department of Anatomy and Histology, Institute for Biomedical Research, The University of Sydney, NSW, April 24th.

(62) Richardson, D.R. (2002) Iron chelators of the pyridoxal isonicotinoyl hydrazone class are effective anti-tumor agents. School of Chemistry, University of Sydney, New South Wales, May 17th.

(63) Richardson, D.R. (2002) Transport of the cyclin-dependent kinase inhibitor, $p21^{WAF1/CIP1}$ to the nucleus is

prevented by potent iron chelators. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 9. *

(64) Kwok, J.C. and **Richardson, D.R.** (2002) Anthracyclines inhibit the intracellular mobilization of iron from ferritin in myocardial and neoplastic cells. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 9. ***Invited Speaker Presentation by Ph.D student J. Kwok.****

(65) Richardson, D.R. (2002) Molecular mechanisms involved in G_1/S arrest after incubation with chelators. Centre for Sickle Cell Disease, Howard University, Washington DC, USA, September 11th .

(66) Kwok, J.C. and **Richardson, D.R.** (2002) Unexpected effects of anthracyclines on cellular iron metabolism. Centre for Sickle Cell Disease, Howard University, Washington DC, USA, September 11th. ***Invited Speaker Presentation by Ph.D student J. Kwok.***

(67) Richardson, D.R. (2002) The use of iron chelators for the treatment of cancer, Friedreich's ataxia and iron overload disease. Australian Society of Anaesthesia Technicians, 6th Annual Educational Conference and Scientific Exhibition, "Development Beyond Technology", 14-16th of November, Sydney. *

(68) Richardson, D.R. (2003) Erythroid differentiation and protoporphyrin IX down-regulate frataxin expression: Characterisation of frataxin expression compared to molecules involved in iron metabolism and hemoglobinisation. Friedreich's Ataxia Research Conference, National Institutes of Health, Bethesda, Maryland, USA. February 15$^{th.}$
Invited Key Participant*

(69) Richardson, D.R. (2003) Development of iron chelators of the PCIH class for the treatment of Friedreich's ataxia: Chelators that bind mitochondrial iron. Friedreich's Ataxia Research Conference, National Institutes of Health, Bethesda, Maryland, USA. February 16th . ***Invited Key Participant***

(70) Chaston, T.B.+ and Richardson, D.R. (2003) Iron chelators of the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone class as potential agents to treat Friedreich's ataxia. Friedreich's Ataxia Research Conference, National Institutes of Health, Bethesda, Maryland, USA. February 16th. ***Invited Speaker Presentation by Ph.D student*** T. Chaston.

(71) Richardson, D.R. (2003) Friedreich's ataxia and mitochondrial iron metabolism and the use of chelators as potential therapies. Centre for Sickle Cell Disease, Howard University, Washington DC, USA, February 14th.

(72) Richardson, D.R. (2003) Anthracyclines affect the intracellular metabolism of iron: Effects on iron-regulatory protein-RNA-binding activity and the storage of iron in ferritin. Lady Davis Institute for Medical Research, Montreal, Quebec, Canada, February 17th.*

(73) Richardson, D.R. (2003) Molecular characterisation of the effects of anthracyclines on cellular iron metabolism in neoplastic cells and cardiomyocytes. Department of Physiology, University of Western Australia, Nedlands, Perth, March 19th.

(74) Richardson, D.R. (2003) Novel iron chelators for the treatment of iron overload disease and cancer. Department of Pathology, University of Melbourne, Grattan st, Parkville, Victoria 3050, April 15th.*

(75) Richardson, D.R. (2003) Iron: The development of novel therapies for the treatment of cancer. University of Canberra, ACT, 28th April.

(76) Richardson, D.R. (2003) Nitric oxide (NO) is a cytotoxic effector molecule produced by macrophages that binds iron: The mechanism of NO-mediated iron release. Australian National University, Canberra, ACT, 29th April.

(77) Richardson, D.R. (2003) Novel mechanisms of action of anthracyclines: Interactions with the iron storage protein, ferritin.University College London, 5 University St, London WC1E 6JJ, July 8th.*

(78) **Richardson, D.R.** (2003) Nitric oxide is a cytotoxic effector molecule that targets intracellular iron: The role of glutathione and energy metabolism in cellular iron mobilization from tumour cells. University of Ulster, Belfast, Northern Ireland, July 10th

(79) **Richardson, D.R.** (2003) Examination of the mechanism of action of potent iron chelators with anti-tumour activity. Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, NSW, 23rd July.

(80) **Richardson, D.R.** (2003) Structure-activity relationships and the development of iron chelators for the treatment of cancer and iron overload disease. Department of Pharmacy, University of Sydney, August 1st.

(81) **Richardson, D.R.** (2003) The regulatory effects of iron on the expression of molecules involved in cell cycle progression. The VIIIth NSW Cell & Developmental Biology Group Meeting, Sydney, NSW, 5th Nov.

(82) **Richardson, D.R.** (2003) Development of iron chelators as effective anti-tumour agents: Examination of their effects on molecules involved in cell cycle control and proliferation. Australian Society of Medical Research National Scientific Conference, Adelaide, South Australia, November 23.*

(83) **Richardson, D.R.** (2003) Iron: Novel pharmacological strategies to selectively inhibit tumour cell growth. Department of Chemistry, Macquarie University, Sydney, NSW, Nov. 27.

(84) **Richardson, D.R.** (2003) Molecular mechanisms involved in the regulation of cell cycle progression by iron: The Development of Novel Iron Chelators as Potent Anti-Proliferative Agents. The International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, Dec. 4-7.*

(85) **Richardson, D.R.** (2003) The development of novel iron chelators as anti-proliferative agents. Examination of their effects on the expression of molecules involved in cell cycle progression. School of Medical Sciences, Dept. of Pharmacology, Faculty of Medicine, University of New South Wales, Dec. 9th.

(86) **Richardson D.R.** (2004) Novel anti-metabolites and their mechanisms of action. Diabetes Transplant Unit, Prince of Wales Hospital, Sydney, Feb. 11th.

(87) **Richardson D.R.** (2004) Novel strategies of inhibiting tumor growth elicit up-regulation of a gene involved in inhibiting metastasis. Australian Nuclear Science and Technology Organisation, Lucas Heights, 25th Feb.

(88) **Richardson D.R.** (2004) Cytotoxic iron chelators, their metal complexes and the mechanisms of anti-tumor activity in tumor cells. School of Science, Food and Horticulture, University of Western Sydney, Campbelltown Campus, Cambelltown, Sydney, NSW, Feb 26th.

(89) **Richardson, D.R.** (2004) The role of Fe in expression of tumor and metastasis suppressor molecules. Institute of Hematology and Blood Transfusion, U nemocnice 1, 128 20, Prague 2, Czech Republic, April 19th.*

(90) **Richardson, D.R.** (2004) Nitric oxide is a cytotoxic effector molecule that induces Fe mobilisation from neoplastic cells: Characterisation of glutathione-dependent transport mechanisms. Department of Pathophysiology, Faculty of Medicine, Charles University, Prague, Czech Republic, April 19th.*

(91) **Richardson, D.R.** (2004) Novel strategies of inhibiting tumour growth by targeting iron and dysregulating cell cycle control molecules and metastasis suppressors. Prince of Wales Hospital Oncology Group, Radiotherapy Seminar Room at 4pm, Level 3 in RadOnc, Sydney, NSW. April 30th.

(92) **Richardson, D.R.** (2004) Design of novel anti-tumor drugs: Iron chelators with potent activity and selectivity. University of New South Wales, School of Chemistry, Sydney, June 9th.

(93) **Richardson, D.R.** and Le, N.T.V. (2004) Dissection of novel molecular mechanisms involved in inhibiting cancer cell proliferation and cell cycle progression by iron depletion. The 7th International Symposium on Chelating Agents in Biomedicine, Toxicology and Therapeutics, Pilsen, Czech Republic, July 8 – 11. *

(94) **Richardson, D.R.** (2004) The development and design of novel and selective anti-tumor agents and their

mechanisms of action: iron chelators for the treatment of cancer. Charles University, Czech Republic, Hradec Karlove, July 13th.

(95) **Richardson, D.R.** (2004) Cellular transport of nitric oxide: A mechanism mediated by iron and the transporter, MRP1. Department of Physiology and Pharmacology, University of New South Wales, Sydney, August 3rd.

(96) **Richardson, D.R.** (2004) Iron, the cell cycle and the metastasis suppressor gene, Ndrg-1: Novel pharmacological mechanisms for inhibiting tumour growth and metastasis. Western Australian Institute for Medical Research, Royal Perth Hospital, Perth, Western Australia, November 5. *

(97) **Richardson, D.R.** (2004) The role of iron in cell cycle progression: Molecular and cellular examination using novel iron chelators. Monash University, Melbourne, Victoria, November 10.*

(98) **Richardson, D.R.** (2004) A novel mechanism of nitric oxide transport: Iron, glutathione and the drug efflux pump, MRP1. Second Australian Health and Medical Research Congress (AHMRC), Sydney, MEPSA Section, November 21-26.

(99) **Richardson, D.R.** (2005) Novel chelators for central system disorders that involve the alterations in the metabolism of iron and other metal ions. Third International Conference on Diet and Optimal Health: A Conference Organised by the Linus Pauling Institute, May 18-25 2005, Hilton Hotel, Portland OR, USA. *

(f-4) ***Invited Academic Lectures***

(1) **Richardson, D.R.** (1992) Proteins involved in the metabolism of iron in mammalian cells. ***Invited Lecture***. Invitation extended by Assoc. Professor Ann English, 3rd year Biochemisty/Chemistry students, Concordia University, Montreal, Québec, Canada, October 15th.

(2) **Richardson, D.R.** (1997) Tumour suppressor proteins and their control of the cell cycle and cellular proliferation. ***Invited Lecture.*** Invitation extended by Assoc. Prof. P. Ladds to lecture to General Pathobiology Course TV3022, James Cook University, 22nd May.

(3) **Richardson, D.R.** (2003) Novel ribonucleotide reductase inhibitors: iron chelators. 3rd year Pharmacology, University of New South Wales, Sydney, October.

(f-5) ***Participation in Conferences – Involvement as Session Chair***

(1) Invited Chair and coordinator of the iron metabolism section of the 6th Internet World Congress for Biomedical Sciences (14-25th February 2000).
(2) Invited Co-chair of the session "Iron Restriction Strategies" at the International Conference on HIV and Iron, March 31st -April 1st, Brugge, Belgium.
(3) Invited Co-chair of the session "Iron and Cancer" at the BioIron World Congress, Cairns, August 18-23rd 2001.
(4) Invited Co-chair of the session "DNA Damage and Cancer" International Conference on Oxidative Pathways in Health and Disease, Sydney, December 2-4th 2001.
(5) Invited Session Chair, European Iron Club, Porto, Portugal, September 25-28, 2002 (invitation declined due to other commitments).
(6) Invited Chair of the session "Metal Ions and Nitric Oxide" Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 15th, 2002.
(7) Invited Chair of Session 8, 13th International Conference on oral chelation in the treatment of thalassaemia and other diseases, Prague, Czech Republic, July 12-15, 2003.
(8) Invited Chair of the session "Regulation of Iron Transport and Metabolism", European Iron Club, Vienna, Austria, Aug 22, 2003
(9) Invited Chair of the session "Oxidative/Nitrosative DNA Damage and Carcinogenesis": International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, December, 2003
(10) Invited Chair of 2 Sessions: Nitric Oxide, Metals and Endothelial Function" and "Iron Metabolism in

Thalassaemia and other Diseases", Fourteenth International Conf. on Oral Chelation, Utrecht, The Netherlands, April 14-17th, 2004

(11) Invited Chair of Session VI, 7th International Symposium on Chelating Agents in Biomedicine, Toxicology and Therapeutics, Pilsen Czech Republic, July 8-11, 2004.

(12) Invited Chair of Session 4 on "Iron Chelation and Cellular toxicity" European Iron Club, France, Sept. 10th 2004

(13) Invited Chair of the Session "Metals in health and disease II" Symposium, AHMRC Congress, Nov. 25, 2004.

(g) POSTGRADUATE AND UNDERGRADUATE TEACHING

(g-1) Postgraduate Research Teaching

Ph.D Students

(1) January 1998 – Dec. 2000: **Principal Supervisor** of Miss Stacey Wardrop (B.Sc. 1st Class Honours), Full-Time Ph.D Student, Dept. of Medicine, U.Q. **Awards:** Year 1 supported by ARC Large grant to D.R.R. Years 2 and 3 supported by a *John Earnshaw Scholarship from the Queensland Cancer Fund - Ranked #1* in this competition. "Top-up" funds provided by D.R. ($2000 pa) in yrs. 2 & 3. **Published:** **(1)** Wardrop and Richardson (1999) ***Eur. J. Biochem.***: **(2)** Wardrop, Watts and Richardson (2000*).* ***Biochemistry***; **(3)** Wardrop and Richardson (2000) ***Eur. J. Biochem.;*** **(4)** Wardrop et al. (2001) ***J. Leuk. Biol.***; **(5)** Watts, Wardrop and **Richardson** (2000) *The Sixth Internet World Congress on Biomedical Sciences.* ***Invited Conference Paper*** ; **** Thesis passed May 2002.*** ***Current Position:*** Research Officer, University of Queensland.

(2) March 1998-to Dec. 2001: **Principal Supervisor** of Miss Erica Becker (B.Sc. 1st Class Honours), Full-Time Ph.D Student. **Awards:** Awarded a *Friedreich's Ataxia Support Group of Queensland Scholarship.* **Published:** **(1)** Becker and Richardson (1999) ***J. Lab. Clin. Med***; **(2)** Richardson, Becker and Bernhardt (1999) ***Acta Cryst. Sect. C***; **(3)** Becker and Richardson (2000) ***Int. J. Biochem. Cell Biol.;*** **(4)** Richardson, Mouralian, Ponka, and Becker (2001) ***Biochim. Biophys. Acta***; **(5)** Becker, Greer, Ponka. Richardson (2002) ***Blood***; **(6)** Becker, Lovejoy, Watts, Richardson. (2000) ***Br. J. Pharmacol;*** **(7)** **International Patent Application:** Richardson, Bernhardt and Becker (2000); **** Thesis passed Feb. 2003.*** ***Current Position:*** Research Officer, QIMR, Qld.

(3) * July 1998-to present: **Principal Supervisor** of Mr. Ralph Watts, **Part-Time Ph.D Student** (Full-Time Research Assistant in my lab supported by ARC Large grant). **Awards:** **(1)** 2003 Travel Grant of $1000 from the Society Free Radical Research Australasia (SFRR(A)) to attend International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan,; **(2)** Awarded Poster Prize at the International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, 2003; **(3)** 2004 Competitive Travel Grant of $2000 from the SFRR(A) to attend the International Society of Free Radical Research, Buenos Aires, Argentina. **Published:** **(1)** Wardrop, Watts and Richardson (2000) ***Biochemistry***; **(2)** Watts and Richardson (2000) ***J. Lab. Clin. Med.***; **(3)** Watts, Wardrop and Richardson (2000) *The Sixth Internet World Congress on Biomedical Sciences.* ***Invited Conference Paper;*** **(4)** Watts and Richardson (2001) ***J. Biol. Chem.***; **(5)** Watts and Richardson (2001) ***Eur. J. Biochem.***; **(6)** Watts, Ponka and Richardson (2003) ***Biochem. J.;*** **(7)** Watts and Richardson (2004) ***Biochim. Biophys Acta. Thesis passed July 2004***

(4) * February 1999-to present: **Principal Supervisor** of Mr David Lovejoy (B.Sc 1st Class Honours), Full-Time Ph.D student (Co-Supervisor: Dr. P. Bernhardt, Dept. Chemistry, Univ. QLD). **Awards**: Supported by a ***University of Sydney Postgraduate Scholarship***. **Published:** **(1)** Lovejoy, Richardson and Bernhardt (2000) ***Acta Cryst. Sect. C***; **(2)** Lovejoy and Richardson (2000) ***Exp. Opin. Invest. Drugs***; **(3)** Lovejoy and Richardson (2002) ***Blood***; **(4)** Becker, Lovejoy, Richardson (2003) ***Br. J. Pharmacol.***; **(5)** Chaston, Lovejoy, Watts and Richardson (2003) ***Clin. Cancer Res.*** ; **(6)** Lovejoy, Gao and Richardson (2000) Metal Ions in Biology and Medicine, Vol. 6, pp221-3. **(7)** Lovejoy, Bernhardt and Richardson (2000) Metal Ions in Biology and Medicine, Vol. 6, pp224-6. **(8)** Lovejoy and Richardson (2003) ms. in preparation. **(9)** Walcourt et al. (2004) ***Int. J. Biochem. Cell Biol***; **(10)** Yuan, Lovejoy and Richardson (2004) ***Blood*** **(11)** Richardson and Lovejoy (2002) Provisional Patent-filed; **(12)** Richardson and Lovejoy (2003) Provisional Patent-filed. **(13)** Richardson and Lovejoy (2003) Provisional Patent-filed. **(14)** Richardson and Lovejoy (2003) Provisional Patent-in preparation. ***Thesis passed April 2004***

(5) * January 2000-to present: **Principal Supervisor** of Miss Juliana Kwok, Full-Time Ph.D student. **Awards:** Supported in Year 1 by a HRI Ph.D Scholarship from DR and in Years 2 and 3 by a competitive *National Heart*

Foundation Ph.D Scholarship. Also awarded a competitive University of Sydney Postgraduate Scholarship. Awarded National Heart Foundation Travel Scholarship 2002. Winner 2002 SFRR(A) Invited Presentation Award. Winner 2003 ASMR (NSW) Eli Lilly Student Prize for Best Oral Presentation by a Student. Awarded Poster Prize at the International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, 2003. **Published: (1)** Kwok and Richardson (2000) ***Redox Report;*** **(2)** Kwok and Richardson (2002) ***Crit. Rev. Hematol. Oncol.*; (3)** Kwok and Richardson (2002) ***Mol. Pharmacol.;*** **(4)** Kwok & Richardson (2003) ***Mol. Pharmacol.;*** **(5)** Kwok & Richardson (2004) ***Mol. Pharmacol.*** **(6)** Wong, Kwok and Richardson (2004) ***Biochim. Biophys. Acta*; (7)** Xi, Wong, Kwok and Richardson (2004) in preparation. ***Thesis passed Oct. 2003.***

(6) January 2001-present: **Principal Supervisor** of Mr. Tim Chaston, Full-Time Ph.D student. **Awards:** *Scholarship awarded by the NSW and Victorian Friedreich's Ataxia Associations.* **Published: (1)** Chaston & Richardson (2003) ***Clin. Cancer Res.;*** **(2)** Chaston & Richardson (2003) ***J. Biol. Inorg. Chem;*** **(3)** Chaston, T. and Richardson, D.R. (2002) ***Am J. Hematol*** **(4)** Bernhardt et al. (2003) ***J. Biol. Inorg. Chem;*** **(5)** Chaston, Watts, Yuan, Richardson (2004) ***Clin Cancer Res. (in press). Thesis submitted May 2004***

(7) January 2001- present. **Principal Supervisor** of Mr. Nghia Le, Full-Time PhD student, HRI and CCIA Ph.D Scholarship from D.R.R. **Published: (1)** Le and Richardson (2002) ***Int. J. Biochem. Cell Biol.;*** **(2)** Le and Richardson (2002) ***Biochim. Biophys. Acta-Reviews in Cancer*; (3)** Le and Richardson (2003) ***Carcinogenesis*** **; (4)** Le and Richardson (2004) ***Biomarkers and Environment*** - Invited Conference Paper. **(5)** Le and Richardson (2004) ***Int. J. Cancer. ;*** **(6)** Le and Richardson (2004) ***Blood.*** **(7)**. ***Thesis submitted September 2004.***

(8) January 2001-present: **Associate Supervisor** of Piao (Alex) Chin (University of Queensland). Principal Supervisor Dr. Paul Bernhardt, Department of Chemistry, University of Queensland: **Awards:** *Awarded a University of Queensland Postgraduate Scholarship. Awarded University of Queensland Travel Scholarship.* **Published: (1)** Bernhardt, Chin, Richardson (2001) ***J. Biol. Inorg. Chem.;*** **(2)** Armstrong et al. (2002) ***Eur. J. Inorg. Chem.;*** **(3)** Bernhardt et al. (2003) ***J. Biol. Inorg. Chem.(4)*** Bernhardt et al. ***J. Chem Soc. Dalton Trans (in press). Thesis passed.***

(9) April 2001-April 2003. **Principal Supervisor** of Michael Food (M.Sc), Full-Time PhD student. **Awards:** Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship. **Published: (1)** Sekyere, Food and Richardson (2002) ***FEBS Lett.***; **(2)** Food Sekyere and Richardson (2002) ***Eur. J. Biochem.;*** **(3)** Food and Richardson (2002) ***Redox Report.***

(10) Nov. 2002-present. **Principal Supervisor** of Louise Dunn (B.Sc 1st Class Honours), Full-Time Ph.D student. **Awards:** Supported by a Ph.D Scholarship from DR in 2003 and *awarded a NHMRC Ph.D Scholarship 2004, 2005; Kumar Award for Best Student Presentation* – MEPSA Annual Meeting, Sydney, 2003; Travel Bursary to attend European Iron Club, France, 2004; **Published:** Sekyere, Dunn, Richardson (2003) ***FEBS Lett*** ; Sekyere, Dunn and Richardson (2004) ***Biochim. Biophys. Acta*** (in press); **In Preparation**: Sekyere et al. 2004 Biochem. J.

(11) March 2004- present. **Principal Supervisor** of Mr. Yohan Suryo Rahmanto (B.Sc. 1st Class Honours), Full Time Ph.D student. **Awards:** Supported by APA Scholarship (UNSW). Awarded a Competititve 2004 Travel Grant of $1000 from the Society Free Radical Research Australasia

(12) Jan 2004 –present. **Principal Supervisor** of Miss Danuta Kalinowski, (B.Sc. 1st Class Honours), ***Pharmacol. Rev*** (in preparation). Full Time Ph.D student. Scholarship from ARC grant.

(13) March 2004-present. **Principal Supervisor** of Mr. Shawn Xiangcong (MSc), Full time Ph.D student. Scholarship from NHF and Cure Cancer grant.

M.Sc Student

(1) October 1992-July 1994: **Co-supervisor** of Dr. Vera Neumannova, Department of Medicine, McGill University, Montreal Quebec, Canada (**Degree Awarded November 1994**). **(see Section E, publications #** 18,20,22,34). (Principal Supervisor: Professor Prem Ponka).

(g-2) Past and Present Undergraduate Research Teaching

(1) September 1992-March 1993: Supervisor of Mr Mathew C. Cheung, Undergraduate Final Year Research Project, Department of Physiology, McGill University, Montreal, Canada. Project: "The role of membrane potential in iron transport from transferrin."

(2) September 1993-March 1994: Supervisor of Miss Daniel Bradshaw, Undergraduate Final Year Research Project, Department of Physiology, McGill University, Montreal, Canada. Project: "The Use of Iron Chelators as Effective Anti-Proliferative Agents against Neuroblastoma Cells.

(3) September 1993-March 1994: Supervisor of Mr Milan Sen, Undergraduate Final Year Research Project, Department of Physiology, McGill University, Montreal, Canada. Project: "The effect of nitrogen monoxide on intracellular iron metabolism of K562 erythroleukemia cells."

(4) January-April 1995: Supervisor of Mr. Eric Amar, Part-time Research Student, Department of Medicine, McGill University, Montreal, Canada. Project: "The Iron Metabolism of Human Neuroblastoma."

(5) June-September 1994: Supervisor of Miss Elise Tran, McGill University Undergraduate, Supported by a Challenge 1994 Summer Studentship. Project: "The Potential of Iron Chelators of the Pyridoxal Isonicotinoyl Hydrazone Class as Effective Anti-Proliferative Agents. **(* Co-author on publication #23-Section E).**

(6) June-September 1995: Supervisor of Miss Lindani Mwase, McGill University Physiology Undergraduate, Supported by a Challenge 1995 Summer Studentship. Project: "The effect of iron chelators at inhibiting tumour cell proliferation."

(7): Supervisor of Mr. Timothy Chaston, University of Canberra Graduate Student (May-Dec. 2000). Mechanisms of iron mobilization from macrophages, the role of ceruloplasmin, cellular activation, and ferroportin1 in iron mobilization.

(8) Supervisor of Visiting Scientist, Mr Lars Hummerich – 8 week Internship within my lab August –September 2000: as part of the teaching program of the Institute of Biochemistry, Justus Liebig University of Giessen (Germany). "The Physiological and Pathophysiological Roles of Melanotransferrin."

(9) Supervisor of Mr. Daniel Johnson, Work Experience in a Research Laboratory as a part of the NSW Secondary School Work Experience Program, December 4-8, 2000.

(10) Supervisor of Mr. Noeris Salam, U. Syd. Molecular Biotechnology Program, (MOBT3002), 1 month research project in my lab, Aug-Sept, 2002. Project Title: Cancer cell growth and iron chelators as anti-tumour agents.

(11) Supervisor of Mr. Luke Stacey, U.Syd. Mol. Biotechnology Program, (MOBT3002), 1 month research project in my lab, Sept-Oct, 2003. Project Title: Development of hydrazide iron chelators for the treatment of iron overload.

(12) Supervisor of Charles Wang, Undergraduate, CCIA Summer Scholarship in my lab, Dec. 2003-March 2004.

(13) Supervisor of Elise Tu, Undergraduate, CCIA Summer Scholarship in my lab, Jan-March 2004.

(14) Supervisor of Maggie Fung, Undergraduate, CCIA Summer Scholarship in my lab, Jan-March2004.

(15) Supervisor of Mr. Yohan Suryo Rahmanto in a 3 month Research Project (entitled: Hyperexpression of Melanotransferrin) in my lab as part of the Diploma of Innovation Management (INOV4301;UNSW). Dec 2003-March 2004.

(16) Supervisor of Mr. Charles Wang, as part of a Friedreich's Ataxia Research Alliance of Australia Undergraduate Scholarship Project, March – May 2004 & part-time researcher June-December 2004

(17) Co-supervisor of Miss Ameena Gilhotra, in a 2 semester Research Project component of the courses "PHAR 4922 Pharmaceutical Chemistry A (Advanced) and PHAR 4925 Pharmaceutical Chemistry B (Advanced), U. Syd. Project title: "Synthesis and Biological Activity of Water-Soluble Iron Chelators as Anti-Cancer Agents"

(18) Supervisor of Mr. Tzu-Hsiang (Peter) Wei, Undergraduate, CCIA Summer Scholarship, Nov. 04-Feb 05.

(19) Supervisor of Mr. Conan Wai Hang Chan, Undergraduate, CCIA Summer Scholarship, Dec. 04-Feb 05.

(20) Co-supervisor of Yi-Tyng Jessie Liao, Undergraduate, Summer Scholarship Dec 04-Feb 05.

(g-3) Summary of Past Undergraduate Teaching (Demonstrating, Tutoring and Lectures)

(1) June 1986-November 1986: Laboratory Demonstrator - Chemistry 142, Chemistry for the Life Sciences, School of Mathematical and Physical Sciences, Murdoch University, Perth, Western Australia.

(2) June 1987- November 1987: Laboratory Demonstrator - 2nd year Biochemistry, School of Biological Sciences, Murdoch University, Perth, Western Australia).

(3) August 1990-December 1990: Part-Time Tutor, Biological Science 112 (Nursing Students), Human Biology/Physiology/Biochemistry (Curtin University, Perth, Western Australia).

(4) September-October 1991: Laboratory Demonstrator, 3rd Year Microbiology, Department of Microbiology, (University of Western Australia, Perth, Western Australia).

(5) Tenurable Lecturer: James Cook University, Department of Physiology and Pharmacology (lecturing, tutoring, preparing exams and marking in nursing and science courses) July 1996 – July 1997.

(6) Tutor: Problem-Based Learning Tutorials First and Second Year Medical Students, Faculty of Medicine, University of Sydney, April 2000-June 2002, Feb-April. 2004 (6 h/week per block).

(g-4) List of Lectures Given

- ***Please note: These series of lectures (1996-1997) were designed by myself. Student Assessments are attached for those courses assessed by the university concerned (See Letters 17-23).***

1996

SC2001:120 Pathophysiological Processes (2nd year Nursing Science students) **5 Lectures** - The Vascular System; **2 Lectures:** The Circulatory System; **2 Lectures:** Lipoprotein Metabolism **2 Lectures**: Haemopoietic System; **1 Tutorial** : Summary and revision of material covered, exam preparation.

NS3502:04 Pathophysiology II (3rd year Nursing Science students) 5 Lectures: The circulatory system

1997

SC1011:03 Physiological Systems and Processes I (1st year Nursing Science): **2 Lectures**: Musculoskeletal Anatomy; **2 Lectures**: Muscles and the Skeleton ; **8 x 2 h Laboratory Practicals + marking of lab assignments**: Anatomy and Histology

Human Pathophysiology and Pharmacology I (SC2011:04) and Pathophysiology I (NS3501:04) (2nd and 3rd year Nursing Science students) **4 Lectures + 2 Tutorials**: Inflammation and anti-inflammatory pharmacology; **2 Lectures + 2 Tutorials**: Antimicrobial Pharmacology ; **3 Lectures + 2 Tutorials**: The biology of cancer

Advanced Physiology PP3101 (3rd year Science Students): **1 Lecture**: Cell Structure; **5 Lectures**: Regulation of Proliferation – Cell Cycle and Neoplasia; **2 Lectures**: Blood (Haematopoiesis, stem cell theory etc)

Chemical Pharmacology PP3150 (3rd yr. Science students): **4 Lectures + 2 Practicals**: Chemotherapy; **3 Lectures**: Inflammation and anti-inflammatory drugs

Invited Lecture (Invitation extended by Assoc. Prof. Phillip Ladds, 22/5/97): General Pathobiology TV3022 (3rd yr. Science students) Tumour Suppressor Proteins and their Control of the Cell Cycle.

Departmental Honours Course (B.Sc. honours students) **2 Lectures and assignment:** Scientific writing etc

(g-5) Undergraduate Teaching – University of Sydney and University of NSW

Problem Based Learning Tutorials for Graduate Medicine Course - University of Sydney.

(1) 2000: Endocrine, Nutrition and Gastroenterology (April-July, 2000), twice per week (6h/week) for 11 weeks.
(2) 2000: Cardiovascular - 1st yr. medical students (July-Sept. 2000), twice/week (6h/week) for 7 weeks
(3) 2001: Block 1, Foundation Block, 1st yr. medical students twice/week (6 h/week) for 10 weeks (Feb-May).
(4) 2001: Block 2, Drug & Alcohol/Musculoskeletal Sciences, 1st yr. medical students, twice per week (6 h/week) for 8 weeks (May-June).
(5) 2001: Block 3, Respiration, 1st yr. medical students, twice per week (6 h/week) for 6 weeks.
(6) 2002: Block 1, Foundation Block, 1st year medical students, twice/week (6 h/week) for 10 weeks (Feb-May)
(7) 2002: Block 2, Drug & Alcohol/Musculoskeletal Sciences, 1st yr. medical students, twice per week (6 h/week) for 8 weeks (May-June).
(8) 2004: Block 1, Foundation Block, 1st yr. medical students twice/week (6 h/week) for 8 weeks (Feb-May).

(g-6) Theses Examined

(1) ***Masters Qualifying Thesis Research Proposals*** (James Cook University, Townsville) by Ms. Melanie J. Williams (7/10/96) and Mr. Michael Ashenden (19/8/96).

(2) ***B.Sc Honours Theses*** (James Cook University, Townsville, **November**, 1996)
(a) Tania Beldan: "Change in matrix metalloproteinase expression during embryo implantation in the rat uterus."
(b) Kylie Wilson: "The effects of halocarbons and anti-oxidants upon cytochrome P450: NADPH P450 reductase complex in rat liver microsomes."
(c) Sharelle Anne Sturgeon: "In vivo assessment of cyanobacteria toxin-induced liver damage in rats."

(3) ***Master of Medical Science*** (University of Queensland, June 1997; External Examiner). Candidate: Dr. Prasutr Thawornchaisit: "The regulation of lymphocyte iron metabolism in haemochromatosis."

(4) ***Doctor of Philosophy*** (University of Queensland, Brisbane, August 1997; Internal Examiner). Candidate: Dr. Stefano Goldwurm: "Isolation and characterisation of haemochromatosis candidate genes."

(5) ***Doctor of Philosophy Introduction and Progress Evaluation***: "Iron : Pathways of Absorption." Mrs. Hayley Inglis (Queensland Institute of Medical Research and Department of Medicine, University of Queensland, July 1998, internal examiner).

(6) ***Doctor of Philosophy*** (University of Western Australia, Perth, December 2000; External Examiner). Candidate: Mr. Anthony Kicic: "The anti-proliferative potential of Fe chelators in the treatment of liver cancer."

(7) ***Doctor of Philosophy*** (University of Sydney, Sydney, October 2000; Internal Examiner). Candidate: Miss Kristine Hardy "The effect of anti-oxidants on signalling pathways and gene expression in T-lymphocytes."

(8) ***Doctor of Philosophy*** (University of Singapore, Singapore, External Examiner, 2003) Candidate: Mr. Julian, Loh Kuan Woei.

(9) ***Doctor of Philosophy*** (University of New South Wales, Internal Examiner, 2003) Candidate: Ms. S.P. Seetulsingh-Goorah "Cytotoxicity induced by extracellular ATP in a human leukaemic cell line: Characteristics and mechanisms."

(10) ***Doctor of Philosophy*** (University of Western Australia, External Examiner, 2004) Candidate Ms. Carla Thomas "The validation and use of the rat instestinal epithelial cell line 6 (IEC6) to study the role of ferroportin and divalent metal ion transporter 1 in the uptake of iron from Fe(II) and Fe(III).

(g-7) Research Staff Taught in my Laboratory

(1) August 1994 - May 1995: Mrs Ania Wilczynska, Senior Research Assistant, Lady Davis Institute for Medical Research (Supported by the Lady Davis Institute for Medical Research and grants from the Medical Research Council of Canada to D.R.R.).

(2) May 1995-May 1996: Miss Kamini Milnes, Research Assistant, Lady Davis Institute for Medical Research (Supported by grants from the Medical Research Council of Canada and National Cancer Institute of Canada to D.R.R.).

(3) June-September 1995: Miss Francoise Lheureux, Research Assistant, Lady Davis Institute for Medical Research (Supported by a grant from the Medical Research Council of Canada to D.R.R.).

(4) January-May 1996: Miss Dominic Granger, Research Assistant, Lady Davis Institute for Medical Research (Supported by a grant from the Medical Research Council of Canada to D.R.R.).

(5) October 1997 – March 1999: Mr. Grant Darnell, Research Assistant, Department of Medicine, University of Queensland (supported by grant #970360 from the NH&MRC to D.R.R.).

(6) January-May 1998: Miss Kristy James, Research Assistant, Department of Medicine, University of Queensland (supported by a Kathleen Cuningham Foundation Breast Cancer Foundation Research Grant to D.R.R.)

(7) February-May 1998: Miss Joanne Hiatt, Research Assistant, Department of Medicine, University of Queensland (supported by a University of Queensland New Staff Grant, ARC Small Grant, and Ramaciotti Foundation Grant to D.R.R.).

(8) June 1998 –December 1998: Miss Ingrid Gausse, Research Assistant, Department of Medicine, University of Queensland (supported by grant #981826 from the NH&MRC to D.R.R.).

(9) July 1998 –Apr. 2004 to present: of Mr. Ralph Watts, Research Assistant, (Part-Time Ph.D Student), Department of Medicine, University of Queensland (supported by an ARC Large grant # A09801140 to D.R.R.).

(10) Feb 2003-Aug 2004: Mr. Ian Napier, Research Assistant, CCIA (Supported by an MDA USA grant)

(11) Feb 2003-Aug 2004: Miss Laura Castelnoble, Research Assistant, CCIA (Supported by NH&MRC grant # 189710 to D.R.R.).

(12) Nov 2003-present: Miss Megan Whitnall, Research Assistant, CCIA (Supported by a UniSearch Commercial Grant to D.R.R.).

(g-8) Postdoctoral Scientists Trained in my Laboratory

(1) October 1995-May 1996: Dr. Christine Boumah, Postdoctoral Fellow, Lady Davis Institute for Medical Research (Supported by a grant from the National Cancer Institute of Canada to D.R.R.).

(2) March-November 1999: Dr. Judith Candy, Postdoctoral Research Officer, Department of Medicine, University of Queensland (supported by grant #981826 from the NH&MRC to D.R.R.).

(3) July 1999 – December 2000: Dr. Jin Gao, Postdoctoral Research Officer, Department of Medicine, University of Queensland and Heart Research Institute, Sydney (supported by grant #970360 from the NH&MRC to D.R.R. and start-up funds from the Heart Research Institute). Appointed NH&MRC Peter Doherty Postdoctoral Research Fellow.

(4) January 2000 - to present: Dr. Eric Sekyere, Postdoctoral Research Officer, The Heart Research Institute, (supported by grant #981826 from the NH&MRC to D.R.R.).

(5) April 2001-April 2003: Dr. Simon Liang, Postdoctoral Research Officer, Heart Research Institute/Childrens Cancer Institute Australia (supported by the National Ataxia Foundation USA and the Heart Research Institute)

(6) March 2002-present: Dr. Cynthia Wong, Postdoctoral Research Officer, Heart Research Institute/ Children's Cancer Institute Australia, Metabolic Pharmaceuticals Contract to D.R.R.

(7) April 2002-present: Dr. Sarah Tandy, Postdoctoral Research Officer, Heart Research Institute/Children's Cancer Institute Australia, (supported by NH&MRC grant # to D.R.R.).

(8) November 2002-October 2003: Dr. Lijuan Xie, Postdoctoral Research Officer, CCIA (supported by MDA USA grant to D.R.R.)

(9) Nov 2002-June 2003: Dr. Jun Yuan, Postdoctoral Research Officer, CCIA (supported by NH&MRC grant # 189710 to D.R.R.).

(10) Jan 2003- Feb 2004 . Juliana Kwok, Postdoctoral Research Officer, Children's Cancer Institute Australia (Supported by National Heart Foundation Research Grant to D.R.R.).

(11) April 2003- present. Dr. Effie Nurtjahja-Tjendraputra, Post-doctoral Research Officer, Childrens Cancer Institute Australia (Supported by CCIA)

(12) August 2003-December 2003: Dr. Xiang-Ju Long, Post-doctoral Research Officer, CCIA (Supported by a 6 month Visiting Roche Post-Doctoral Scholarship).

(13) August 2003-present: Dr. Jenny Park, Post-doctoral Research Officer, CCIA, Supported by a NHMRC Project Grant.

(14) October 2003-present: Dr. Fu Dong, Post-doctoral Research Officer, CCIA (supported by NH&MRC grant # 189710 to D.R.R.).

(15) January 2004-present: Dr. Jonathan Howard, Post-doctoral Research Officer, CCIA, supported by a UniSearch Commercial Grant to D.R.R.

(16) January 2004-present: Dr. Neil Davies, Post-doctoral Research Officer, supported by a NSW Leuk. Found. Grant to D.R.R. Awarded a Travel Bursary to attend the European Iron Club Conference, France, 2004.

(17) May 2004 - to present: of Dr. Ralph Watts, Postdoctoral Research Officer, Supported by ARC Discovery grant.

(g-9) Miscellaneous Teaching Responsibilities

(1) Faculty of Medicine representative on the panel conducting interviews for the selection of students into the University of Sydney Medical Program (24-28th September 2001).

(2) Personal and Professional Development Interviews for Medical Students at the Uni. of Sydney (2001, 2002).

(3) Heart Research Institute: Examination of yearly Ph.D progress reports and panel interview of all students (2000-2001).

(4) Member of the Board of Postgraduate Studies in Dentistry, Medicine and Pharmacy – Postgraduate Coordinator, Heart Research Institute. January-June 2002

(5) Case Coordinator, Problem-Based Learning, Faculty of Medicine, University of Sydney, March 2002.

(6) Part-time supervision of Honours project of Mr. Anthony Ryan (Centenary Institute, University of Sydney). Development of Chelators as Anti-Mycobacterial Agents, 2002.

(7) Supervisor of Mr. Noeris Salam, Student in Industry Placement Scheme in the Molecular Biotechnology Program (MOBT3002), Uni. Sydney, Aug-Sept. 2002 (1 month research project).

(8) Post-graduate student review panel, Faculty of Medicine, School of Women's and Children's Health, UNSW, 2002-present.

(9) Supervisor of Mr. Luke Stacey, Student in Industry Placement Scheme in the Molecular Biotechnology Program (MOBT3002), Uni. Sydney, Aug-Sept. 2003 (1 month research project).

(10) Two day course taken (Nov. 2003) for being a Facilitator in the new UNSW Undergraduate Medical Program

(g-10) Projected Involvement in Teaching

Over the next 5 years I will be involved in being a facilitator/tutor (6h/week for 16 weeks/yr) for medical students in the Faculty of Medicine, UNSW. In addition, I hope to continue to attract excellent Ph.D students to my lab so that their number remains constant at 5-6. I am also undertaking the teaching of graduate students and undergraduate students (summer scholarship students and specific courses eg. Mol. Biotech Program, USyd) in my lab in order to stimulate interest in furthering their careers particularly at the MSc. and Ph.D levels.

(g-11) Visiting Scientists to my Lab

(1) Lorraine Caldwell, Department of Chemistry, Univ. Queensland, Oct 2002
(2) Assistant Professor XiMing Yuan, Linkoping University, Sweden, Feb-March 2003
(3) Alex Chin, Department of Chemistry, Univ. Queensland, April and May 2004
(4) Dr. Persson Lennart, Linkoping University, Sweden, Dec 2003-Feb 2004

(h) ADMINISTRATIVE RESPONSIBILITIES/COMMITTEES/CONTRIBUTIONS TO INSTITUTION/MANAGEMENT OF THE INSTITUTION

(1) Financial management of my research grants (1994-present).
(2) Various research-related administrative functions (preparation and typing of grant proposals, animal research committee applications, research reports on completed grants etc).
(3) Department of Medicine, Royal Brisbane Hospital Research Committee (February 1998-November 1999)
(4) Management, Direction and Development of Institional Policy at the Heart Research Institute via the combined Group Leader Meetings with the Executive Director every month (November 1999 - present)
(5) Publication and Grant Assessment Committee (PGAC) of the Heart Research Institute for manuscript and grant submissions (November 1999-present).
(6) Elected Treasurer (2000-2003) of the Society for Free Radical Research Australasia
(7) Member of the "Space" Committee, The Heart Research Institute (2000-2001).
(8) Seminars to members of the public regarding recruitment to the Heart Research Institute Endowment Fund (2001-present)
(9) CRC Compendium Bid (Biometals) - Group Leader of the Metabolic and Circulatory Systems Research

Section 2000

(10) Editorial Board Member for the *International Journal for Biochemistry and Cell Biology and* Molecules in Focus Editor (*2002 Impact factor = 3.3*). Involves reviewing and commissioning review articles for my "Molecules in Focus" section (October 2000-to present).

(11) Radiation Safety Committee, The Heart Research Institute (May 2001-June 2002)

(12) Editorial Board Member for *Redox Report* (*Impact Factor = 1.6*; May 2001-present)

(13) Member of the Occupational Health and Safety Committee, The Heart Research Institute (November 2001-June 2002).

(14) Seminars to private institutions (eg. Rotary Club of Sydney) regarding the Heart Research Institute's aims and medical research teams (Development of donations to the institute from private organisations) November 2001-present.

(15) Management, Direction and Development of Institutional Policy at the Children's Cancer Research Institute via the Research Management and Institute Management Committees with the Executive Director every month (July 2002 - present)

(16) Postgraduate Student Assessment Committee, Sydney Children's Hospital (2002-present).

(17) Editorial Board Member for *J. Lab. Clin. Med* (*Impact Factor = 2; May 2001-present*)

(18) Interview Committee, assessment of applicants for Research Officer and Senior Research Officer Positions, Sydney Children's Hospital, September 2003.

(19) Faculty Research Grants Committee, UNSW, Oct-Nov. 2003, 2004

(20) Editorial Board Member of *BioMetals* (*Jan 2004-present*)

(21) Chairman of the Radiation Safety Committee, Children's Cancer Institute Australia, March 2004-presen

(22) Editorial Board Member of the *Biochemical Journal* (Impact Factor = 4.5; March 2004-present)

(23) NHMRC Fellowship Mock Interview Panel, UNSW, Sydney, 2004

(24) Member of the Executive Committee of the Mutagenesis and Experimental Pathology Society of Australia, March 2004-present

(25) Editorial Board Member of *Expert Opinion in Investigational Drugs* (Impact Factor = 3.1; Sept 2004-present)

(26) Editorial Board Member of the *Journal of Inorganic Biochemistry* (Impact Factor = 2.3; Nov. 2004-present)

(27) Invited Presentation on NHMRC grant writing by the University of NSW, Research Office, Dec. 14, 2004

(i) PEER REVIEW

(i-1) Granting Agencies:

(i) Special Emphasis Review Panel, National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID), October 28-30, 1998 – Review of 17 program project (PO1) applications (NIAID-DMID-98-004).
(ii) The Israel Science Foundation – Israel Academy of Sciences and Humanities, 1998.
(iii) Australian Research Council – Large Grant Scheme, 1998,1999, 2000, 2001.
(iv) The Royal Children's Hospital Foundation, Herston, Queensland, 1998.
(v) National Health and Medical Research Council of Australia, 1999, 2000. 2003, 2004
(vi) Australian Research Council - Research Fellowship Scheme, 2000.
(vii) Dutch Cancer Society 2001, The Netherlands
(viii) The National Heart Foundation of Australia, 2002
(ix) Welcome Trust, United Kingdom, 2002, 2003
(x) National Ataxia Foundation, United Kingdom, 2003.

(i-2) Journals:

I review 120-150 manuscripts per year for *Cell, Science, Lancet, Cancer Cell, Nature Reviews Drug Discovery, BLOOD, Circulation, Cancer Res., Exp. Cell Res., Am. J. Pathology, Mol. Pharmacol., Neuroscience, Biochem. J., J. Leuk. Biol., Biochem. J., FEBS Letters, Int. J. Cancer, Eur. J. Biochem., J. Hepatology, Brit. J. Haematol., Microbiology, Neuroscience Research, Biochimica Biophysica Acta, Am. J. Hematol., Mitochondrion, J. Inorg. Biochem, J. Neurochem., Redox Report, J. Invest. Dermatol., Cancer Chemotherapy and Pharmacology, Biochem. Pharmacol., Proteomics, Clinical Sci., Free Radical Res., Free Rad. Biol. Med., J. Lab. Clin. Med., Mitochondrion, J. Invest. Med., Pharmacol Res, Cancer Immunology Immunotherapy, Exp. Opin. Invest. Drugs, BioMed Central-Clinical Pharmacology, Int. J. Biochem. Cell Biol., J. Agric. Food Chem., BioMetals, Drug Safety, Clinical and Experimental Pharmacology and Physiology*

(j) SCIENTIFIC DISCIPLINE INVOLVEMENT

(j-1) Scientific Societies

(i) Astronomical Society of Western Australia (Junior member: 1974-1977)
(ii) Australian Society for Medical Research (ASMR; 1987-1989; 1996-to present)
(iii) The Australian Physiological and Pharmacological Society (APPS 1997-to present)
(iv) The American Society of Hematology (January 1999-present)
(v) Society for Free Radical Research Australasia (December 1999- present)
(vi) Sydney Free Radical Society Inc. (December 2000-present)
(vii) National Association of Research Fellows (December 2001-present).
(viii) Mutagenesis and Experimental Pathology Society of Australia (November 2002- present)
(ix) Australian and New Zealand Society for Cell and Developmental Biology (November 2003-present)

(j-2) Organisation of Local, National and International Meetings

(i) Organiser and Convenor of the 1998 AMP/ASMR Queensland Biomedical Research Awards, Queensland Institute of Medical Research, Westpac Auditorium, June 2nd, 1998.
(ii) Invited Coordinator and Chairman of the Symposium on Iron Metabolism - the 6th Internet World Congress for Biomedical Sciences (INABIS 2000), February 14-25th , 2000 (*www.uclm.es/inabis2000/symposia).*
(iii) Invited Member of the Organising and Scientific Committees of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, Australia.
(iv) Organiser of the Society of Free Radical Research Australasia Meeting 2002, Wollongong, Australia.
(v) Invited Member of the Scientific Committee, 7th International Symposium on Chelating Agents in Biomedicine, Toxicology and Therapeutics, Pilsen, Czech Republic, July 8-11, 2004.
(vi) Invited to organize a Symposium on Metal Ions in Health and Disease for the MEPSA/AHMRC Congress in Sydney (November 21-26, 2004)
(vii) Organiser of the Friedreich's Ataxia in New South Wales Research Meeting, Sydney, July 31st, 2004.
(viii) Invited Member of the Organising Committee, 15th International Conference on Oral Iron Chelators, Taiwan, 2005.

(k) EDITORIAL BOARD MEMBERSHIP

(i) Editorial Board Member for the ***International Journal for Biochemistry and Cell Biology*** and Molecules in Focus Editor (Impact factor = 3.6). Involves full editorial review, examining papers and commissioning review articles for the "Molecules in Focus" section (October 2000-to present).
(ii) Editorial Board Member for ***Redox Report*** (June 2000-present) Impact Factor = 1.6; May 2001-present)
(iii) Editorial Board Member of the ***Journal of Laboratory and Clinical Medicine*** (Impact Factor = 2.0; June 2003-present)
(iv) Editorial Board Member of ***BioMetals*** (Impact Factor = 2.6; Jan. 2004-present)
(v) Editorial Board Member of the ***Biochemical Journal*** (Impact Factor = 4.4; March 2004-present)
(vi) Editorial Board Member of ***Expert Opinion in Investigational Drugs*** (Impact Factor = 3.1; Sept 2004-present)
(vii) Editorial Board Member of the ***Journal of Inorganic Biochemistry*** (Impact Factor = 2.3; Nov. 2004-present)

Yearly editorial board meetings are are organised at International venues each year to discuss such issues as strategies to increase impact factor, page limit, advertisement of the journal.etc Each of these is paid for by the journal concerned eg. IJBCC (June 2003), BJ (July 2004).

(l) INVOLVEMENT IN THE WIDER COMMUNITY

(1) Interviewed by Mr Peter Mitchell, ABC radio (4QN; Townsville), regarding the 1997 AMP/ASMR Biomedical Research Awards and the significance of the 1997 Medical Research Week in Brisbane, June 6th 1997.

(2) Co-organiser and discussion leader of the Department of Medicine/Queensland Institute of Medical Research Iron Metabolism Journal Club (held every 2nd week; November 1997-November 1998).

(3) Convenor of the Department of Medicine, Central Clinical School, Royal Brisbane Hospital Research Seminar Series (March 1999-Nov. 1999).

(4) Invited lay research seminar given to the Queensland Friedreich's ataxia support group (April 8th 1999) regarding the possible role of iron in the development of the disease and vthe use of iron chelators for therapy.

(5) Invited lay research seminar given to the Friedreichs Ataxia Association of New South Wales (Golden Wing Club, Sydney Airport, Sydney, NSW, August 25th) regarding mitochondrial iron overload in the development of Friedreich's ataxia and the potential of novel chelators to prevent mitochondrial Fe overload.

(6) Invited lay letter regarding our research work on Friedreich's ataxia (FA) to the FA Associations of New South Wales and Queensland (Vol. 1, Feb. 1999). Port and accommodation to these meetings is fully paid for by these journals.

(7) Research article published by the Frieich's Ataxia Association of New South Wales and Queensland entitled "Development and Potential of Novel Iron Chelators for the Treatment of Friedreich's Ataxia: Iron Binding Drugs which Permeate the Mitochondrion." (see Ataxia Australia Issue 3, December 1999 and *www.faa.org.au*).

(8) Invited lay research seminar given to the Thalassemia Centre of New South Wales (Queen Mary Hospital, Sydney, New South Wales,12th January) on new orally effective iron chelators for the treatment of β-thalassemia.

(9) Invited lay research article published by the Thalassemia Society of New South Wales in their Newsletter "Development of a new class of iron chelators for the treatment of β-thalassemia: A long road but light at the end of the tunnel." February 2000.

(10) Invited Co-chair of the session "Iron Restriction Strategies" at the International Conference on HIV and Iron, March 31st -April 1st, Brugge, Belgium.

(11) Invited lay article summarising research presented at the The 10th International Conference on Oral Chelators, Cyprus, 22-26th of March. Newsletter of the Thalassemia Society of New South Wales, April-May 2000.

(12) Invited lay research seminar: "Development of Novel Iron Chelators for the Treatment of β-Thalassemia" given to the New South Wales Thalassemia Group, Concord Hospital, Microbiology Seminar Room, May 22nd, 2000

(13) Invited lay research seminar: Mitochondrial iron overload in the pathogenesis of Friedreich's ataxia and the use of iron chelators as agents to treat this disease. Friedreich's Ataxia Association of New South Wales Meeting, Concord Hospital, Sydney, New South Wales, August 12th, 2000

(14) **Richardson, D.R.** (2000) Lay community awareness booklet on "Iron and the Heart: A Research Report", August 2000, The Heart Research Institute, Sydney, Australia.

(15) Invited lay research seminar on the development of orally effective chelators given to the Australian Thalassemia Association, Central New South Wales Coast, October 28th, 2000

(16) Invited lay research article published by the New South Wales Thalassemia Group 'The Development of Novel Iron Chelators for the Treatment of β-Thalassemia."

(17) Invited lay research article published in the Newsletter of the Friedreich's Ataxia Society of New South Wales 2000 "Iron: Its Role in Friedreich's Ataxia."

(18) Invited lay review article by Becker, E. and **Richardson, D.R.** (2000) Iron: What Role Does it Play in the Pathogenesis of Friedreich's Ataxia. Published on the web site of the Friedreich's Ataxia Association of New South Wales, June 2004.

(19) Research Update Article: **Richardson, D.R.** and Chaston, T. (2001) New Iron Chelators – Iron Binding Drugs with the Potential to Treat Friedreich's Ataxia. In the Newsletter of the Friedreich's Ataxia Association of New South Wales, March 2001 issue, p.4.

(20) Member of the judging panel for the "Johnson & Johnson Research Postdoctoral Prize" ASMR (NSW) Scientific Meeting on June 4th 2001 at the Scientia, University of New South Wales.

(21) Faculty of Medicine representative on the panel conducting interviews for the selection of students into the University of Sydney Medical Program (24-28th September 2001).

(22) Organisation of the Annual General Meeting of the Freidreich's Ataxia Association of NSW at the Heart Research Institute, August 18th 2001 and Children's Cancer Research Institute, August 10th 2002.

(23) Invited lay research presentations by D.R. and my Ph.D student Tim Chaston to the Friedreich's Ataxia Association of NSW, August 18th 2001, August 10th 2002, August 16th 2003.

(24) Personal and Professional Development Interviews for Medical Students at the University of Sydney (2001 & 2002). Interviews conducted by Science Communication Students from UNSW on scientific publications.

(25) Research Update Article: Chaston, T. and **Richardson, D.R.** (2001) The Frataxin Knockout Mouse - A New Model for Screening Drugs that may be Useful for Treating Friedreich's Ataxia (FA). In the Newsletter of the Friedreich's Ataxia Associaon of New South Wales, November 2001 issue.

(26) Seminar to the Rotary Club of Sydney regarding the Heart Research Institute's aims and medical research teams (Development of capital donations to the institute from private organisations). November 2001

(27) Report for the Friedreich's Ataxia Association (FAN) entitled "Progress on Iron Chelators" by E. Becker and **D.R. Richardson**, July 2002 and posted on the FAN website (www.fan.org.au).

(28) Research Report published in the National Ataxia Foundation USA Newsletter (2002) by D.R. Richardson entitled "Friedreich's ataxia and mitochondrial iron overload: The role of frataxin in iron metabolism and the use of novel iron chelators as therapeutic agents."

(29) Childrens Cancer Institute Australia representative at fund raising events including "Luke Greening Memorial Golf Day" Nov 2002 and 2004 and Coates Hire Sydney and Newcastle, "Melbourne Cup Day Fundraising Lunch" Nov 2nd 2003 and 2004

(30) Invited Member of the Judging Panel for TOW Awards (Open Junior Division), November 7 2003, October 22 2004

(31) Invited Member of the Judging Panel for Travel Awards from the Society of Free Radical Research Australasia – October 2003, March 2004, September 2004

(32) Invited Research Update Article for Friedreich's Ataxia Research Association of Australia Newsletter Issue 1, August 2004 by D.R. Richardson entitled: "Progress on Understanding the Role of Iron in Friedreich's Ataxia and the Development of Potential Iron Chelators for Treatment"

(33) Judging panel for reviewing abstracts for oral or poster presentation, Australian Health and Medical Research Congress (AHMRC), MEPSA Section, Sydney, 2004.

(m) PREVIOUS, CURRENT, AND PROPOSED RESEARCH

All the research completed by the applicant has been devoted to the fields of iron (Fe) chelation therapy and Fe metabolism, and hence, it is directly relevant to the current research proposal. This research was based on the development of Fe chelators for clinical use, and on understanding the molecular mechanisms of Fe uptake by cells and the processes involved in intracellular Fe metabolism.

Development of Iron Chelators of the Pyridoxal Isonicotinoyl Hydrazone Class for Clinical Use:

(A.) ***Iron Chelators for the Treatment of Iron Overload Disease:*** I identified 3 novel Fe chelators of the pyridoxal isonicotinoyl hydrazone (PIH) class (PIH analogues) that showed very high activity in a variety of biological assays (publications 1,8,73,79,80,82). These compounds were more effective than the drug in current clinical use, desferrioxamine (DFO), and showed high potential for Fe chelation therapy. I also discovered that these ligands are mainly neutral at pH 7.4 allowing access to intracellular Fe pools (5). Further studies showed that these chelators were highly specific for Fe(III) and had low affinity for other biologically important cations, supporting their high potential for Fe chelation therapy (3). However, these chelators were susceptible to acid-catalysed hydrolysis (relevant as orally effective chelators should be resistant to acid-catalysed hydrolysis in the stomach), and for oral adminstration these drugs should be provided with an enteric coating (2). I also discovered that the most effective chelators had a partition coefficient close to 1, and these data will be important for the design of even more efficient analogues (16,19). Recently, we have synthesized and provisionally patented a novel series of aroylhydrazone chelators that show high Fe chelation activity and low anti-proliferative activity and hold great promise (43,67,90).

(B.) ***Iron Chelators as Anti-Tumour Agents:*** The high chelation activity of the PIH analogues stimulated my interest to investigate their effect on the aggressive childhood cancer neuroblastoma (NB). This tumour is highly sensitive to DFO, and clinical trials have demonstrated that this chelator can markedly affect tumour growth without significant side effects. My studies demonstrated that 3 PIH analogues dramatically reduced Fe uptake from transferrin (Tf) and increased Fe release from NB cells (17). In addition, these ligands were more effective than DFO or PIH at reducing ^{3}H-thymidine uptake (17). Further studies investigated 36 analogues of PIH for their anti-proliferative effect (23). Analogues derived from pyridoxal benzoyl hydrazone showed high Fe chelation activity but low anti-proliferative activity, characteristics that make them appropriate to treat Fe overload. In contrast, analogues derived from salicylaldehyde and 2-hydroxy-1-naphthylaldehyde were effective anti-proliferative agents (as assessed by ^{3}H-thymidine incorporation and cell growth studies) and displayed high Fe chelation activity, properties of chelators suitable to treat cancer (23). More recent work has extensively characterised the 5 most effective PIH analogues as anti-proliferative agents and these studies have demonstrated that the ligands have comparable activity to the cytotoxic agents bleomycin and cis-platin (28). Moreover, the intracellular site of chelator action was identified and experiments demonstrated that the chelators caused apoptosis in some cell types but not others (28). Additional studies examined chelator transport through the cell membrane (29). This work showed that in contrast to PIH which was transported by an energy- and temperature-dependent process, the closely related PIH analogues mobilized Fe from cells by a temperature-dependent but energy-independent mechanism (29). Using X-ray crystallography (42) we have solved the crystal structure of one of the most active anti-tumour chelators identified, namely 311 and its Fe(III) complex. This was important in order to obtain further insight into its high anti-tumour activity. Solution electrochemistry showed that the trivalent oxidation state was dominant over a wide potential range (42). The fact that the Fe(III) complex cannot cycle between the Fe(II) and Fe(III) states indicated that generation of free radicals was not part of this ligand's cytotoxic action. This is supported by culture experiments showing that the addition of Fe(III) to 311 prevented its anti-proliferative effects (42).

To further understand the mechanism of the anti-proliferative action of Fe chelators we have examined the molecular targets of 311 and DFO (41). These studies were done to understand the mechanisms involved in the anti-tumour activity of Fe chelators. Like DFO, 311 increased the RNA-binding activity of the iron-regulatory proteins (IRPs), that are key regulators of cellular Fe metabolism. However, despite the far greater Fe chelation efficacy of 311 compared to DFO, a similar increase in IRP-RNA-binding activity occurred and this was not inhibited by cycloheximide. Further studies examined the effect of 311 and DFO on the expression of p53-transactivated genes crucial for cell cycle control and DNA repair, namely WAF1 and GADD45. DFO and 311 caused a pronounced concentration-dependent increase in the expression of GADD45 and WAF1 mRNA. In accordance with the anti-proliferative effects of DFO and 311, much higher concentrations of DFO (150 μM) than 311 (2.5-5 μM) were required to markedly increase GADD45 and WAF1 mRNA levels. In contrast to the chelators, the Fe(III) complexes of DFO and 311 had no effect on increasing GADD45 and WAF1 mRNA levels, suggesting that Fe chelation was required. The increase in GADD45 and WAF1

mRNA levels appeared to occur by a p53-independent pathway. Since both GADD45 and WAF1 are very important in cell cycle arrest, our results demonstrate for the first time that the increase in the expression of these molecules may play important roles in the anti-proliferative effects of Fe chelators (41). Studies have progressed to examine the molecular mechanisms involved in the inhibition of cell cycle progression mediated by Fe chelators (70).

(2) The Molecular Mechanisms Involved in Iron Metabolism and Transport:

(A.) ***The Role of Melanotransferrin (p97) in Iron Uptake of Melanoma Cells:*** The Fe metabolism of the human melanoma cell was of particular interest as these cells express high concentrations of the membrane-bound transferrin (Tf) homologue, melanotransferrin (MTf), the function of which was unknown. Studies were designed to investigate the relative importance of the 2 putative Fe uptake pathways in melanoma cells, namely, Fe uptake from Tf by receptor-mediated endocytosis (RME), and Fe uptake mediated by MTf. My studies demonstrated for the first time that Fe uptake from Tf occurred by 2 processes, consistent with RME and adsorptive pinocytosis (4,9,15). In addition, I was the first to demonstrate a membrane-bound Fe binding component consistent with MTf (4,6,7). Further work investigated the uptake of inorganic ^{59}Fe complexes by melanoma cells (7), since the MTf Fe-binding site can bind this form of Fe. Iron uptake from inorganic Fe complexes was far greater than that from Tf and occurred by a process that was independent of the RME of Tf (7). Studies using MoAb 96.5 against MTf (but not control MoAbs) showed that this antibody could modulate internalized Fe uptake from low M_r complexes. However, from the extent of the internalization of Fe, MTf appeared to play little role in Fe uptake in this cell line (7). Experiments using CHO cells transfected with the full-length MTf sequence convincingly showed that MTf can transport Fe from low M_r Fe complexes (21). This work was the first to demonstrate that a membrane-bound protein apart from the Tf receptor (TfR) was involved in Fe uptake (21). Obviously, transfection of cells with MTf resulted in a cell model where the molecule was overexpressed at nearly 4 times that seen in the human melanoma SK-Mel-28 cell line. Our more recent studies (51) in SK-Mel-28 cells demonstrated that cleavage of MTf from the cell surface has only a minor effect on Fe uptake by the cell, suggesting that under physiological circumstances MTf plays little role in Fe transport. Interestingly, our studies have shown that MTf is expressed in a wide variety of tissues and it may have a function independent of Fe transport. To definitively investigate the function of MTf we have cloned and mapped the mouse gene and are progressing towards developing a knockout animal.

(B.) ***Molecular Mechanisms of Cellular Iron Metabolism:*** I discovered a novel Fe uptake pathway which was stimulated by incubation of cells with Fe complexes such as ferric ammonium citrate (FAC; 9,24,64). Exposure of cells to DFO resulted in up-regulation of the TfR, whereas exposure to FAC resulted in down-regulation (9). Paradoxically, at Tf concentrations greater than that required for saturation of the TfR, FAC markedly stimulated Fe uptake without increasing Tf uptake (9,24). Other work has demonstrated that the FAC-stimulated Fe uptake process is mediated by a novel Fe transport pathway (24). This novel Fe uptake system may be an important protective mechanism to guard against oxidant stress generated in the presence of small M_r complexes (24). There is little known about the intracellular fate of Fe, and considering this we examined the intracellular form of Fe using Mössbauer spectroscopy (12). These experiments were the first to identify low-spin and high-spin Fe(II) complexes not identified in other cell types (12). Further studies were the first to demonstrate an intracellular pool of Fe free from Tf that could be released by temperature-dependent mechanical wounding (10,26). We have also examined intermediates of Fe uptake in cells using the haem synthesis inhibitor succinylacetone (25). These studies were the first to identify intermediates involved in the transport of Fe to the mitochondrion for haem synthesis (25).

(C.) ***Effect of Redox Species of Nitrogen Monoxide on Tumour Cell Iron Metabolism:*** More recently we have examined the effect of nitrogen monoxide (NO) on Fe metabolism (18,22,49,54,56,65). These studies were initiated as NO derived from macrophages is thought to mobilise Fe from tumour target cells, resulting in cytostasis. We showed that NO could prevent Fe uptake from Tf into cells (18). Further studies examined the effect of NO on the iron-regulatory protein 1 (IRP1; which coordinately stabilises TfR mRNA and inhibits ferritin mRNA translation) and TfR expression (22). Different redox species of NO had differential effects on IRP activation that may have important eregulatory roles (22). In recent studies we have been able to demonstrate for the first time that NO can activate IRP1 by two independent mechanisms, namely direct coordination of the molecule to the FeS centre and also by depleting the cells of Fe (49). We also discovered that the newly identified Fe transporter Nramp2 is not regulated by Fe or NO at the mRNA level like the transferrin receptor, despite the presence of an IRE in its 3' untranslated region (40). Finally, we have discovered that there is a novel link between energy metabolism and NO-mediated iron mobilization (65).

SECTION D - RESEARCH SUPPORT
(a) Past and Present Support

(1) D.R. Richardson (Sole Investigator): ***Medical Research Council of Canada Research Grant*** ("The Iron Metabolism of Human Neuroblastoma" 3 year grant 1995-1998; $86,659 yr. 1, $70,400 yrs. 2 and 3, TOTAL VALUE: $227,459). ***** This grant was ranked #1 by the MRC Cell Physiology Committee.**

(2) D.R. Richardson (Sole Investigator): ***National Cancer Institute of Canada Terry Fox New Investigator Research Grant*** ("The Role of Iron and Secreted Ferritin in the Pathogenesis of Human Neuroblastoma," 3 year grant 1995-1998, $79,533/year, TOTAL VALUE: $238,599). **Letter attached see Section C (m) (letters 6 and 7).**

(3) D.R. Richardson (Sole Investigator): ***National Cancer Institute of Canada Terry Fox Equipment Grant for New Investigators***, "The Role of Iron and Secreted Ferritin in the Pathogenesis of Human Neuroblastoma," $12,319 (1995-1996).

(4) D.R. Richardson (Sole Investigator): ***1994 Challenge Summer Studentship*** for Miss Elise Tran ("The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents", Undergraduate Research Studentship $1000-1500, Federal Government of Canada).

(5) D.R. Richardson (Sole Investigator): ***1995 Challenge Summer Studentship*** for Miss Lindani Mwase ("The effect of iron chelators at inhibiting tumor cell proliferation," Undergraduate Research Studentship $1000-1500, Federal Government of Canada).

(6) P. Ponka and **D.R. Richardson**: ***Medical Research Council of Canada Research Grant*** ("The Effect of Redox-Related Species of Nitrogen Monoxide on Iron Metabolism," 3 yr grant, 1996-1999, $75,757/year, TOTAL VALUE: $227,271).

(7) D.R. Richardson (Sole Investigator): ***Australian Research Council Small Grant*** – James Cook University ("The Effect of Redox Species of Nitrogen Monoxide on Intracellular Iron Metabolism 3 year grant 1997-1999, 1997 $23,000; 1998 $10,000; 1999 $10,000, TOTAL VALUE: $53,000).

(8) D.R. Richardson (Sole Investigator): ***Prestige Research Grant*** - James Cook University ("The Role of the Membrane Tumour Antigen, Melanotransferrin (MTf), in Iron Uptake by the Human Malignant Melanoma Cell", 1997 $46,753).

(9) D.R. Richardson (Sole Investigator): ***Kathleen Cuningham Foundation Breast Cancer Research Grant*** ("The Role of Iron-Transport Molecules in the Pathogenesis of Human Breast Cancer and the Use of Iron Chelators for Treatment, 1997 $52,258; 1998 $54,171, TOTAL VALUE: $106,429).

(10) D.R. Richardson (Sole Investigator): ***National Health and Medical Research Council (NH&MRC) Project Grant*** ("The Iron Metabolism of Human Neuroblastoma (grant #970360)," 3 year grant, 1997-1999: 1997 $72,146; 1998 $58,887; 1999 $62,954, TOTAL VALUE: $193,987).

(11) D.R. Richardson (Sole Investigator): ***Australian Research Council (ARC) Small Grant*** ("A model to examine the defect in human haemochromatosis: Kupffer cells and macrophages from the β-2-microglobulin knockout mouse", 1998: $24,000).

(12) D.R. Richardson (Sole Investigator): ***University of Queensland New Staff Research Grant*** ("Characterisation of a Membrane-Bound Iron Transport Mechanism in Cells that is Stimulated by Extracellular Iron Complexes" 1998: $16,364).

(13) D.R. Richardson (Sole Investigator) : *Queensland Cancer Fund* ("The Role of the Membrane-Bound Tumor Antigen, Melanotransferrin (MTf), in Iron Uptake by Human Malignant Melanoma Cells" 1998-1999). Grant awarded but deferred due to the success of the same project in the NH&MRC competition (see below).

(14) **D.R. Richardson (Sole Investigator):** ***National Health and Medical Research Council (NH&MRC) Project Grant*** ("The Role of the Tumour Antigen, Melanotransferrin, in Iron Uptake by Human Melanoma Cells (grant #981826)" 3 year grant, 1998-2000: 1998 $56,178; 1999 $59,216; 2000 $59,565, TOTAL VALUE: $174,959).

(15) **D.R. Richardson (Sole Investigator):** ***Ramaciotti Foundation Research Grant*** ("The Iron Metabolism of Kupffer Cells and Macrophages from β-2-Microglobulin Knockout Mice: A Model to Examine the Metabolic Defect in Hemochromatosis" 1998: $13,000).

(16) **D.R. Richardson (Sole Investigator):** ***Australian Research Council (ARC) Large Grant*** ("The Effect of Redox-Related Species of Nitrogen Monoxide on Intracellular Iron Metabolism" 3 year grant (application # A09801140), 1998-2000: 1998 $55,000; 1999 $52,000; 2000 $53,500, TOTAL VALUE: $160,500).

(17) M. Appleyard, G.J. Anderson, and **D.R. Richardson**: ***Royal Brisbane Hospital Research Foundation*** 1998 "The Role of the Iron Transporter Encoded by *Nramp2* in the Pathogenesis of the Human Iron Overload Disease Haemochromatosis: $10,000).

(18) G.J. Anderson and **Richardson, D.R.** ***Friedreich's Ataxia Support Group of Queensland*** ("The Role of Frataxin in Cellular Iron Metabolism," 1998: $30,000).

(19) **Richardson, D.R.** ***Ph.D Scholarship from the Friedreich's Ataxia Support Group of Queensland*** for Miss Erica Becker ("The Role of Mitochondrial Iron Overload in the Pathogenesis of Friedreich's Ataxia: Examination of the Function of Frataxin in Mitochondrial Iron and Haem Metabolism, 1998, $25,000; 1999, $25,000; 2000, $25,000).

(20) **Richardson, D.R**. **(Sole Investigator)** ***Equipment Grant Award, Friedreich's Ataxia Support Group of Queensland*** for the project "The Role of Mitochondrial Iron Overload in the Pathogenesis of Friedreich's Ataxia: Examination of the Function of Frataxin in Mitochondrial Iron and Haem Metabolism." October 1998, $5000.

(21) Anderson, G., **Richardson, D.R.** and MacMillan, J. ***The Royal Childrens Hospital Research Foundation***, Royal Brisbane Hospital, "Iron Homeostasis in cellular models of Friedreich's Ataxia," Jan. - Dec. 1999, $33,497.

(22) **Richardson, D.R. (Sole Investigator):** ***National Health and Medical Research Council (NH&MRC) Project Grant*** ("The Physiological and Pathophysiological Roles Melanotransferrin (grant #151620)" 3 year grant, 2001: $75,000; 2002 $75,000; 2003 $75,000 TOTAL VALUE: $225,000.

(23) **Richardson, D.R. (Sole Investigator):** ***Australian Research Council Large Grant***: " The Effect of Nitrogen Monoxide on Intracellular Iron Metabolism (grant # A00106419)," 2001 $63,000; 2002 $60,100; 2003 $58,250, TOTAL VALUE: $181,350.

(24) **Richardson, D.R. (Sole Investigator):** ***National Ataxia Foundation USA Research Grant***: "Friedreich's ataxia and mitochondrial iron overload: The role of frataxin in iron metabolism and the use of novel iron chelators as therapeutic agents. 2001: $62,500.

(25) Davies MJ., Dean, R.J. and **Richardson, D.R**: ***National Health and Medical Research Council (NH&MRC) Project Grant*** ("The role of transition metal ions and redox activity in the development of atherosclerotic plaque ; grant #151630)" 3 year grant, 2001: $65,000; 2002 $65,000; 2003 $65,000 TOTAL VALUE: $195,000.

(26) **Richardson, D.R.** ***Ph.D Scholarship from The Friedreich's Ataxia Association of New South Wales and Victoria for Mr. Tim Chaston***, 2001 $25,000; 2002 $25,000; 2003 $25,000.

(27) **Richardson, D.R. (Sole Investigator):** ***National Health and Medical Research Council (NH&MRC) Project Grant*** ("Potential Anti-Tumour Agents: Iron Chelators of the Pyridoxal Isonicotinoyl Hydrazone Class"; grant # 189710) 3 year grant, 2002 $100,000; 2003 $100,000; 2004 $100,000. TOTAL VALUE: $300,000

(28) **D.R. Richardson (Principal Investigator) and M. Davies:** ***National Health and Medical Research Council (NH&MRC) Project Grant*** ("Mitochondrial Iron Overload and Friedreich's Ataxia: The Role of Frataxin in Iron and Haem Metabolism"; grant # 189720) 3 year grant, 2002 $95,000; 2003 $95,000; 2004 $95,000. TOTAL VALUE: $285,000

(29) **D.R. Richardson: (Sole Investigator):** ***National Heart Foundation Grant***: ("The Iron Metabolism of Myocardial Cells and the Use of Novel Iron Chelators as Potential Agents for the Treatment of Cardiotoxicity Induced by Iron Overload and Anthracyclines", grant # G 01S 0407) 2 year grant: 2003 $47,654; 2004 $42,727. TOTAL VALUE: $90,381.

(30) **D.R. Richardson: (Sole Investigator):** ***Muscular Dystrophy Association USA*:** (The Role of Iron in Friedreich's Ataxia and the Use of Iron Chelation Therapy). 3 year grant 2002-2004: 2002 $66,182, US$: 2003 $68,720, US$; 2004 $69,660, US$.

(31) **Richardson, D.R. (Sole Investigator):** ***National Health and Medical Research Council (NH&MRC) Project Grant*** ("The Physiological and Pathophysiological Roles Melanotransferrin (grant # 300457)" 3 year grant, 2004: $141,750; 2005 $131,750; 2006 $131,750. TOTAL VALUE: $405,000.

(32) **Richardson, D.R. (Sole Investigator):** ***Australian Research Council Discovery Grant***: "The Effect of Nitrogen Monoxide on Intracellular Iron Metabolism," 2004-2006: $85,000pa; TOTAL VALUE: $255,000 **DP0450213**

(33) Bernhardt, P.V. and **Richardson, D.R.:** ***Australian Research Council Discovery Grant***: "Chemical and Biochemical Characterisation of Novel Iron Chelators with Therapeutic Potential" DP0450001 2004-2006: 2004: $100,000, 2005: $90,000, 2006: $90,000 TOTAL VALUE: $280,000. **DP0450001**

(34) Kwok, J.C. and **Richardson, D.R.** ***Cure Cancer Australia Foundation*** "Development of Novel Metal Complexes as Anti-Tumour Agents" 2004: $60,000

(35) Davies, M. and **Richardson, D.R.** ***National Heart Foundation Grant:*** "Are elevated levels of iron and matrix oxidation products present in human atherosclerotic lesions that are prone to rupture?" 2004: $50,000; 2005: $50,000. TOTAL VALUE: $100,000.

(36) **Richardson, D.R.** ***Friedreich's Ataxia Research Alliance, USA.*** Iron chelation efficacy of novel PCIH iron chelators in vivo and their ability to prevent the pathology observed in the conditional frataxin knockout mouse." 2004-2006, $30,000 US pa

(37) ***Seed Funding from ARC for an ARC Network Grant 2004,*** "Metals in Medicine" $30,000. Awarded by ARC to a network of 30 key investigators including **D.R. Richardson** to develop an ARC Network Grant proposal.

(38) **Richardson, D.R.** and Lock, R.B. **(Principal Investigator)** ***Leukaemia Foundation of NSW Grant*** "Novel complexes of gallium as potent anti-leukaemia and lymphoma agents. 2004: $80,000; 2005: $80,000; 2006: $80,000.

(39) **Richardson, D.R.** Friedreich's Ataxia Research Alliance of Australia Undergraduate Scholarship Project, Iron chelators for the treatment of Friedreich's ataxia. $2500 for a research project by undergraduate Mr. Charles Wang.

(40) **Richardson, D.R.** and Davies, M. **(Principal Investigator)** ***National Health and Medical Research Council*** "Mitochondrial Iron Overload and Friedreich's Ataxia: The Role of Frataxin in Iron and Haem Metabolism" Project Grant #350874 2004: $149,250; 2005: $149,250; 2006: $149,250

(41) Dong, F. and **Richardson, D.R.** Iron-Transport Molecules and their Role in the Pathogenesis of Human Breast Cancer and the Use of Novel Iron Chelators as Therapeutic Agents. ***Cure Cancer Australia Foundation*** 2005: $60,000

(b) Grants from Commercial Organisations

(42) **D.R. Richardson**, Metabolic Pharmaceuticals, The Development of Iron Chelators for Clinical Use. TOTAL VALUE $396,000 2002-2004

(43) **D.R. Richardson,** UniSearch, Characterisation and Development of the DpT Iron Chelators as Anti-Tumour Agents. TOTAL VALUE $145,000 2003-2004

SECTION E – PUBLICATIONS

(1) **Richardson, D.R.** (1984) The potential of analogues of pyridoxal isonicotinoyl hydrazone in iron chelation therapy: Assessment in the fetal hepatocyte screen. M.Sc. Preliminary Thesis, Department of Physiology, University of Western Australia, Nedlands, Perth, Western Australia, 6009.

(2) **Richardson, D.R.** (1987) Biochemical evaluation of the potential of analogues of pyridoxal isonicotinoyl hydrazone in iron chelation therapy. M.Sc. Thesis, Department of Physiology, University of Western Australia, Nedlands, Perth, Western Australia, 6009.

(3) **Richardson, D.R.** (1985) Occupational health hazards in the aluminium smelting industry. ***Invited Literature Review***, Worker's Information and Research Centre, Fremantle, Perth, Western Australia, pp 1-55.

(4) **Richardson, D.R.**, Baker, E., Ponka, P., Wilairat, P., Vitolo, M.L. and Webb, J. (1988) Effect of pyridoxal isonicotinoyl hydrazone (PIH) and analogues on iron metabolism in hepatocytes and macrophages in culture. ***Birth Defects*** 23(5B): 81-88.

(5) Ponka, P., **Richardson, D.**, Baker, E., Schulman, H.M. and Edward, J.T. (1988) Effect of pyridoxal isonicotinoyl hydrazone (PIH) and other hydrazones on iron release from macrophages, reticulocytes and hepatocytes. ***Biochim. Biophys. Acta*** 967: 122-129.

(6) **Richardson, D.R.**, Wis Vitolo, M.L., Baker, E. and Webb, J. (1989) Pyridoxal isonicotinoyl hydrazone and analogues. Study of their stability in acidic, neutral and basic solutions by ultraviolet-visible spectrophotometry. ***Biol. Metals*** 2: 69-76.
6
(7) **Richardson, D.R.**, Hefter, G.T., May, P.M., Webb, J. and Baker, E. (1989) Iron chelators of the pyridoxal isonicotinoyl hydrazone class III. Formation constants with calcium(II), magnesium(II) and zinc(II). ***Biol. Metals*** 2: 161-167.

(8) **Richardson, D.R.** (1990) The mechanisms of iron and transferrin uptake by the human malignant melanoma cell. Ph.D. Thesis, Department of Physiology, University of Western Australia, Nedlands, Perth, Western Australia, 6009.

(9) **Richardson, D.R.** and Baker, E. (1990) The uptake of iron and transferrin by the human malignant melanoma cell. ***Biochim. Biophys. Acta*** 1053: 1-12.

(10) **Richardson, D.R.**, Wis Vitolo, L.M., Hefter, G.T., May, P.M., Clare, B.W., Webb, J. and Wilairat, P. (1990) Iron chelators of the pyridoxal isonicotinoyl hydrazone class Part I. Ionization characteristics of the ligands and their relevance to biological properties. ***Inorg. Chim. Acta*** 170: 165-170.

(11) **Richardson, D.R.** and Baker, E. (1991) The release of iron and transferrin by the human malignant melanoma cell. ***Biochim. Biophys. Acta*** 1091: 294-302.

(12) **Richardson, D.R.** and Baker, E. (1991) The uptake of inorganic iron complexes by human melanoma cells. ***Biochim. Biophys. Acta*** 1093: 20-28.

(13) Baker, E., **Richardson, D.R.**, Gross, S. and Ponka, P. (1992) Evaluation of the iron chelation potential of hydrazones of pyridoxal, salicylaldehyde and 2-hydroxy-1-naphthylaldehyde using the hepatocyte in culture. ***Hepatology*** 15: 492-501.

(14) **Richardson, D.R.** and Baker, E. (1992) Two mechanisms of iron uptake from transferrin by melanoma cells. The effect of desferrioxamine and ferric ammonium citrate. ***J. Biol. Chem.*** 267: 13972-13979.

(15) **Richardson, D.R.** and Baker, E. (1992) Intermediate steps in cellular iron uptake from transferrin. Detection of a cytoplasmic pool of iron free of transferrin. ***J. Biol. Chem.*** 267: 21384-21389.

(16) **Richardson, D.R.** and Baker, E. (1992) The effect of desferrioxamine and ferric ammonium citrate on the uptake

of iron by the membrane iron-binding component of human melanoma cells. ***Biochim. Biophys. Acta*** 1103: 275-280.

(17) St. Pierre, T.G., **Richardson, D.R.**, Baker, E. and Webb, J. (1992) A low-spin iron complex in human melanoma and rat hepatoma cells and a high-spin iron(II) complex in rat hepatoma cells. ***Biochim. Biophys. Acta*** 1135: 154-158.

(18) **Richardson, D.R.**, Cameron, K., Robinson, B. and Turner, K.J. (1993) The mechanisms of IgE uptake by human alveolar macrophages and a B-lymphoblastoid cell line (Wil-2wt). ***Immunology*** 79: 305-311.

(19) **Richardson, D.R.**, Ponka, P. and Baker, E. (1994) The effect of the iron(III) chelator, desferrioxamine, on iron and transferrin uptake by the human malignant melanoma cell. ***Cancer Res.*** 54: 685-689.

(20) **Richardson, D.R.** and Baker, E. (1994) Two saturable mechanisms of iron uptake from transferrin in human melanoma cells. The effect of transferrin concentration, chelators and metabolic probes on transferrin and iron uptake. ***J. Cell. Physiol.*** 161: 160-168.

(21) Ponka, P., **Richardson, D.R.**, Edward, J.T. and Chubb, F.L. (1994) Iron chelators of the pyridoxal isonicotinoyl hydrazone class. Relationship of the lipophilicity of the apochelator to its ability to remove iron from reticulocytes *in vitro*. ***Can. J. Physiol. Pharmacol.*** 72: 659-666.

(22) **Richardson, D.R.** and Ponka, P. (1994) The iron metabolism of the human neuroblastoma cell. Lack of relationship between the efficacy of iron chelation and the inhibition of DNA synthesis. ***J. Lab. Clin. Med.*** 124: 660-671.

(23) **Richardson, D.R.**, Neumannova, V. and Ponka, P. (1995) Nitrogen monoxide decreases iron uptake from transferrin but does not mobilise iron from prelabelled neoplastic cells. ***Biochim. Biophys. Acta*** 1266: 250-260.

(24) Edward, J.T., Ponka, P. and **Richardson, D.R.** (1995) Lipophilicity of the ligands and iron(III) complexes of analogues of pyridoxal isonicotinoyl hydrazone. Relationship to biological activity. ***BioMetals*** 8: 209-217.

(25) Neumannova, V., **Richardson, D.R.**, Kriegerbeckova, K. and Kovar, J. (1995) Growth of human tumor cell lines in transferrin-free, low-iron medium. ***In Vitro*** 31: 625-632.

(26) Kennard, M., ***Richardson, D.R.**, Gabathuler, R., Ponka, P. and Jefferies, W.A. (1995) A novel iron uptake mechanism mediated by GPI-anchored human p97. ***EMBO J.*** 14: 4178-4186. **** As stated on the title page of this article, the contribution of D.R.R. was of equal importance to that of M.K.***

(27) **Richardson, D.R.**, Neumannova, V., Nagy, E. and Ponka, P. (1995) Effects of nitrogen monoxide species on cellular proliferation and transferrin and iron uptake by erythroleukemia (K562) cells. ***Blood*** 86: 3211-3219.

(28) **Richardson, D.R.**, Tran, E.H., and Ponka, P. (1995) The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents. ***Blood*** 86: 4295-4306.

(29) **Richardson, D.R.** and Ponka, P. (1995) Identification of a mechanism of iron uptake which is stimulated by hydroxyl radicals generated via the iron-catalysed Haber Weiss reaction. ***Biochim. Biophys. Acta – Mol. Cell. Res.*** 1269: 105-114.

(30) **Richardson, D.R.**, Ponka, P. and Vyoral, D. (1996) Distribution of iron in reticulocytes after inhibition of heme synthesis with succinylacetone. Examination of the intermediates involved in iron metabolism. ***Blood*** 87: 3477-3488.

(31) **Richardson, D.R.**, Dickson, L. and Baker, E. (1996) Intermediate steps in cellular iron uptake from transferrin. II. A Cytoplasmic pool of iron is released from cells via temperature-dependent mechanical wounding. ***In Vitro*** 32: 486-495.

(32) **Richardson, D.R.** and Ponka, P. (1996). Effects of Nitrogen Monoxide on Cellular Iron Metabolism. ***Methods in Neurosciences*** Vol. 31, Chapter 29, pp 329-345 (Maines, M., ed.), Academic Press.

(33) **Richardson, D.R.** and Ponka, P. (1997) The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. ***Biochim. Biophys. Acta - Rev. Biomembranes*** 1331: 1-40.

(34) **Richardson, D.R.** and Milnes, K. (1997) The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents II. The mechanism of action of ligands derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone. ***Blood*** 89: 3025-3038.

(35) **Richardson, D.R.** (1997) Mobilization of iron from neoplastic cells by some iron chelators is an energy-dependent process. ***Biochim. Biophys. Acta - Bioenergetics*** 1320: 45-57

(36) **Richardson, D.R.** (1997) Cytotoxic analogues of the iron(III) chelator pyridoxal isonicotinoyl hydrazone: Effect of complexation with copper(II), gallium(III), and iron(III) on their anti-proliferative activity. ***Antimicrob. Agents Chemother.*** 41: 2061-2063.

(37) **Richardson, D.R.** (1997) Iron chelators as effective anti-proliferative agents. ***Can. J. Physiol. Pharmacol.*** 75: 164-1180.

(38) Ponka, P. and **Richardson, D.R.** (1997) Can ferritin provide iron for hemoglobin synthesis ? ***Blood*** 89: 2611-2613.

(39) **Richardson, D.R.** and Ponka, P. (1998) Orally effective iron chelators for the treatment of iron overload disease: The case for a further look at pyridoxal isonicotinoyl hydrazone (PIH) and its analogs. ***J. Lab. Clin. Med.*** 132:351-352.

(40) **Richardson, D.R.** and Richardson, V. (1998) The effect of ferricyanide and other impermeable oxidants on the growth of human neoplastic cells. ***In Vitro*** 34(1): 30-34.

(41) Ponka, P., Beaumont, C. and **Richardson, D.R.** (1998) Function and regulation of transferrin and ferritin. ***Semin. Hematol.*** 35(1): 35-54.

(42) **Richardson, D.R.** and Ponka, P. (1998) Pyridoxal isonicotinoyl hydrazone and its analogues: Potential orally effective iron-chelating agents for the treatment of iron overload disease. ***J. Lab. Clin. Med.*** 131:306-315. (***an editorial relevant to this article appears on p 290 of this issue of the journal***).

(43) **Richardson, D.R.** (1998) Analogues of pyridoxal isonicotinoyl hydrazone (PIH) as potential iron chelators for the treatment of neoplasia. ***Leukemia and Lymphoma*** 31:47-60.

(44) **Richardson, D.R.** and Ponka, P (1998) The development of iron chelators to treat iron overload disease and their use as experimental tools to probe intracellular iron metabolism. ***Am. J. Hematol.*** 58:299-305.

(45) **Richardson, D.R.**, Chua, A. and Baker, E. (1999) Activation of an iron-transport mechanism from transferrin in hepatocytes by preincubation with low molecular weight iron complexes. ***J. Lab. Clin. Med.*** 133:144-151.

(46) Wardrop, S.L. and **Richardson, D.R.** (1999) The effect of intracellular iron concentration and nitrogen monoxide on Nramp2 expression and non-transferrin-bound iron uptake. ***Eur. J. Biochem.*** 263:41-49.

(47) Darnell G. and **Richardson, D.R.** (1999) The potential of analogues of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents III: The effect of the ligands on molecular targets involved in proliferation. ***Blood*** 94:781-792.

(48) **Richardson, D.R.** and Bernhardt, P. (1999) Crystal and molecular structure of 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone (NIH) and its iron(III) complex: An iron chelator with anti-proliferative activity. ***J. Biol. Inorg. Chem.*** 4:266-273.

(49) Becker, E. and **Richardson, D.R.** (1999) Development of novel aroylhydrazone ligands for iron chelation therapy: The 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH) analogues. ***J. Lab. Clin. Med.*** 134:510-521.

(50) **Richardson, D.R.** (1999) The role of ceruloplasmin and ascorbate in cellular iron release. ***J. Lab.Clin. Med.*** 134:454-465. (***an editorial relevant to this article appears on p 431 of this issue of the journal***).

(51) **Richardson, D.R.** (1999) Therapeutic potential of iron chelators. ***Exp. Opin. Invest. Drugs.*** 8(12):2141-2158. ***Invited Review.***

(52) **Richardson, D.R.**, Becker, E. and Bernhardt, P.V. (1999) The biologically active iron chelators 2-pyridylcarboxaldehyde isonicotinoyl hydrazone, 2-pyridylcarboxaldehyde benzoyl hydrazone and 2-furfural isonicotinoyl hydrazone. ***Acta Crystallographica Section C*** C55:2102-2105

(53) Lovejoy, D., **Richardson, D.R.** and Bernhardt, P.V. (2000) X-ray crystal structure of the chelator 2-hydroxy-1-naphthylaldehyde-2-methyl-3-thiosemicarbazone. ***Acta Crystallographica Section C*** C56:341-342.

(54) Wardrop, S.L., Watts, R. and **Richardson, D.R.** (2000) Nitrogen monoxide (NO) activates IRP-RNA binding by two possible mechanisms – an effect on the Fe-S cluster and iron release from cells. ***Biochemistry*** 39, 2748-2758.

(55) **Richardson, D.R.** (2000) The tenth international conference on oral iron chelators in the treatment of β-thalassemia and other diseases and biomed meeting. ***Exp. Opin. Invest. Drugs*** 9:1151-1154.

(56) **Richardson, D.R.** (2000) The role of melanotransferrin (tumor antigen p97) in iron uptake by the human malignant melanoma cell. ***Eur. J. Biochem.*** 267:1290-1298.

(57) Lovejoy, D.B. and **Richardson, D.R.** (2000) Complexes of gallium(III) and other metal ions and their potential in the treatment of neoplasia. ***Exp. Opin. Invest. Drugs*** 9:1257-1270. ***Invited Review.***

(58) **Richardson, D.R.** (2000) Another jigsaw piece towards solving the iron metabolism puzzle: The cloning of the iron exporter, ferroportin 1. ***Redox Report*** 5(1):7-9. ***Invited Commentary***

(59) Watts, R.N. and **Richardson, D.R.** (2000) Examination of the mechanism of action of nitrogen monoxide on iron uptake from transferrin. ***J. Lab. Clin. Med.*** 136:149-156.

(60) Kwok, J. and **Richardson, D.R.** (2000) The cardioprotective effect of the iron chelator dexrazoxane (ICRF-187) on anthracycline-mediated cardiotoxicity. ***Redox Report*** 5(6):317-324.

(61) Wardrop, S.L. and **Richardson, D.R.** (2000) Interferon-γ and lipopolysaccharide regulate the expression of Nramp2 and increase the uptake of iron from low relative molecular mass complexes by macrophages. ***Eur. J. Biochem.*** 267:6586-6593.

(62) Sekyere, E. and **Richardson, D.R.** (2000) The membrane-bound transferrin homologue melanotransferrin: Roles other than iron transport ? ***FEBS Lett.*** 483:11-16.

(63) **Richardson, D.R.** (2001) The use of iron chelators as anti-cancer agents. In: Iron Chelation Therapy, Ed. C. Hershko. ***Invited Chapter***

(64) **Richardson, D.R.** and Dean, R.T. (2001) Does free extracellular iron exist in haemochromatosis and other pathologies, and is it redox active ? ***Clin. Sci.*** 100:237-238. ***Invited Editorial***

(65) Becker, E. and **Richardson, D.R.** (2001) Frataxin: Its role in iron metabolism and the pathogenesis of Friedreich's ataxia. ***Int. J. Biochem. Cell Biol.*** 33:1-10.

(66) **Richardson, D.R.** (2001) Proton transfer and ferredoxin I: The secrets of redox-driven proton transfer between solvent and a buried iron-sulphur cluster ([3Fe-4S]). ***Redox Report*** 6:11-13 ***Invited Commentary***

(67) **Richardson, D.R.** (2001) The controversial role of deferiprone in the treatment of thalassemia. ***J. Lab. Clin. Med.*** 137:324-329.

(68) **Richardson, D.R.** (2001) Iron and gallium increase iron uptake from transferrin by human melanoma cells: Further examination of the ferric ammonium citrate-activated iron uptake process. ***Biochim. Biophys. Acta*** 1536, 43-54.

(69) **Richardson, D.R.**, Mouralian, C., Ponka, P. and Becker E. (2001) Development of potential iron chelators for the treatment of Friedreich's ataxia: Ligands that mobilize mitochondrial iron. ***Biochim. Biophys. Acta*** 1536, 133-140.

(70) Watts, R.N. and **Richardson, D.R.** (2001) Nitrogen monoxide (NO) and glucose: Unexpected links that affect intracellular iron metabolism and iron mobilisation. ***J. Biol. Chem.*** 276:4724-4732.

(71) Thomas, S. Houta, Hunt, N., **Richardson, D.R.**, Stocker, R. (2001) Postranslational regulation by pyrrolidine dithiocarbamate of indoleamine 2,3-dioxygenase in interferon-γ activated human mononuclear phagocytes. ***J. Immunol.*** 166:6332-6340.

(72) Gao, J. and **Richardson, D.R.** (2001) The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents IV. The mechanisms involved in inhibiting cell cycle progression. ***Blood*** 98, 842-850.

(73) **Richardson, D.R.** (2001) Identification of the ferrireductase that may be involved in iron transport in the small intestine: Revisting Crane's controversial oxidoreductase. ***Redox Report*** 6(3):133-135. ***Invited Commentary***

(74) Bernhardt, P.V., Chin, P. and **Richardson, D.R.** (2001) Unprecedented oxidation of a biologically active aroylhydrazone chelator catalysed by iron(III): Serendipitous identification of diacylhydrazone ligands with high iron chelation efficacy. ***J. Biol. Inorg. Chem.*** 6:801-809

(75) Green, D.A., Antholine, W.E., Wong, S.J., **Richardson, D.R**. and Chitambar, C.R. (2001) Ribonucleotide reductase as a preferential target for the inhibition of leukemic cell growth by 311, a novel iron chelator of the pyridoxal isonicotinoyl hydrazone class. ***Clin. Cancer Res.*** 7:3574-3579.

(76) **Richardson, D.R.** (2001) The Development of Iron Chelators for Clinical Use and the Molecular Mechanisms of the Metabolism and Transport of Iron in Normal and Neoplastic Cells. D.Sc. Thesis, The University of Western Australia, Perth, Australia.

(77) Le, N. and **Richardson, D.R.** (2002) Ferroportin1: A candidate iron export molecule. ***Int. J. Biochem. Cell Biol.*** 34:103-108.

(78) Wardrop, S.L., Wells, C., Ravasi, T., Hume, D.A., and **Richardson, D.R.** (2002) Induction of Nramp2 in activated mouse macrophages is dissociated from regulation of the Nramp1, classical inflammatory genes and genes involved in iron metabolism. ***J. Leuk. Biol.*** 71:99-106.

(79) **Richardson, D.R.** (2002) Iron uptake by neoplastic cells. In: Cell and Molecular Biology of Iron Transport, Marcel Dekker Publishers, Chapter 21, pp 509-538. ***Invited Chapter***

(80) Sekyere, E., Food, M. and **Richardson, D.R.** (2002) A second melanotransferrin gene (MTf2) and a novel protein isoform: Explanation for the membrane-bound and soluble forms of melanotransferrin? ***FEBS Lett*** 512, 350-352.

(81) Kwok, J. and **Richardson, D.R.** (2002) The iron metabolism of neoplastic cells: Alterations that facilitate proliferation ? ***Crit. Rev. Oncol. Hematol.*** 42(1):65-78. ***Invited Review***

(82) **Richardson, D.R.** (2002) Iron chelators as therapeutic agents for the treatment of cancer. ***Crit. Rev. Oncol. Hematol.*** 42(3):267-281. ***Invited Review***

(83) Becker, E., Greer, J., Ponka, P. and **Richardson, D.R.** (2002) Erythroid differentiation and protoporphyrin IX down-regulate frataxin expression in Friend cells: Characterisation of frataxin expression compared to molecules involved in iron metabolism and hemoglobinisation. ***Blood*** 99:3813-3822.

(84) Lovejoy, D.B. and **Richardson, D.R.** (2002) Novel "hybrid" iron chelators derived from aroylhydrazones and thiosemicarbazones demonstrate high anti-proliferative activity that is selective for tumor cells. ***Blood*** 100:666-676.

(85) Watts, R.N. and **Richardson, D.R.** (2002) The mechanism of nitrogen monoxide (NO)-mediated iron mobilization from cells: NO intercepts iron before incorporation into ferritin and indirectly mobilizes iron from ferritin in a glutathione-dependent manner. ***Eur. J. Biochem.*** 269: 3383-3392.

(86) Le, N. and **Richardson, D.R.** (2002) The role of iron in cell cycle progression and the proliferation of neoplastic cells. ***Biochim. Biophys. Acta - Reviews in Cancer*** 1603: 31-46.

(87) **Richardson, D.R.** (2002) Deferiprone: Greater efficacy at depleting myocardial than hepatic iron ? ***Lancet*** 360: 501-502. ***(Invited Commentary).***

(88) Food, M., Sekyere, E.O. and **Richardson, D.R**. (2002) The soluble form of the membrane-bound transferrin homologue, melanotransferrin, inefficiently donates iron to cells via non-specific internalisation and degradation of the protein. ***Eur. J. Biochem.*** 269:4435-4445.

(89) Kwok, J. and **Richardson, D.R.** (2002) Unexpected effects of doxorubicin on iron regulatory protein-RNA-binding activity: The iron and copper complexes of anthracyclines decrease RNA-binding activity. ***Mol. Pharmacol.*** 62: 889-901

(90) Chaston, T.B. and **Richardson, D.R.** (2003) Redox chemistry and DNA interactions of the 2-pyridyl-carboxaldehyde isonicotinoyl hydrazone class of iron chelators: Implications for toxicity in the treatment of iron overload disease. ***J. Biol. Inorg. Chem.*** 8:427-438.

(91) Chaston, T., Lovejoy, D., Watts, R.N., **Richardson, D.R.** (2003) Examination of the Anti-Proliferative Activity of Iron Chelators: Multiple Cellular Targets and the Different Mechanism of Action of Triapine compared to Desferrioxamine and the Potent PIH Analogue 311. ***Clin. Cancer Res.*** 9(1), 402-414.

(92) **Richardson, D.R.** (2003) The role of hypoxia and nitric oxide in the regulation of cellular iron metabolism. ***J. Lab. Clin. Med.*** 141(5):289-291. ***(Invited Editorial)***

(93) Becker, E., Lovejoy, D.B., Greer, J., Watts, R., and **Richardson, D.R.** (2003). Novel aroylhydrazone iron chelators differ in their iron chelation efficacy and anti-proliferative activity: Identification of a new class of potential anti-proliferative agents. ***Br. J. Pharmacol.*** 138: 819-830.

(94) Watts, R.N., Ponka, P. and **Richardson, D.R.** (2003) Effects of nitrogen monoxide and carbon monoxide on molecular and cellular iron metabolism: Mirror-image effector molecules that target iron. ***Biochem. J.*** 369, 429-440.

(95) Lovejoy, D. and **Richardson, D.R.** (2003) Iron chelators as anti-neoplastic agents: Current developments and promise of the PIH class of chelators. ***Curr. Med. Chem.*** 10, 1065-1078. ***(Invited Review)***

(96) Armstrong, C.M., Bernhardt, P.V, Chin, P. and **Richardson, D.R.** (2003) Structural variations and formation constants of first row transition metal complexes of biologically active aroylhydrazones. ***Eur. J. Inorg. Chem.*** 1145-1156.

(97) **Richardson, D.R.** (2003) Friedreich's ataxia: Iron chelation as a therapeutic strategy ? ***Exp. Opin. Invest. Drugs.*** 12(2), 235-245. ***(Invited Review)***

(98) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines induce accumulation of ferritin iron in normal and neoplastic cells: Inhibition of the ferritin Fe mobilization pathway. ***Mol. Pharmacol.*** 63:849-861.

(99) Wong, C. and **Richardson, D.R.** (2003) β-Thalassaemia: Emergence of new and improved chelators for treatment. ***Int. J. Biochem. Cell Biol.*** 35(7):1144-1149.

(100) Chaston, T.B. and **Richardson, D.R.** (2003) Iron chelators for the treatment of iron overload disease: The relationship between structure, redox activity and toxicity. ***Am. J. Hematol.*** 73:200-210. ***(Invited Review)***

(101) Le, N.T.V. and **Richardson, D.R.** (2003) Potent iron chelators increase the mRNA levels of the universal cyclin-dependent kinase inhibitor, $p21^{WAF1/CIP1}$, but paradoxically inhibit translation: A potential mechanism of cell cycle dysregulation. ***Carcinogenesis*** 24:1045-1058.

(102) Sekyere, E., Dunn, L. and **Richardson, D.R.** (2003) The double-edged nature of using genetic databases: Melanotransferrin genes and transcripts. ***FEBS Lett.*** 547:233

(103) **Richardson, D.R**. (2003) The biogenesis of [Fe-S] clusters: The role of the unorthodox ABC ATPase, SufC. ***Redox Report*** 8(3):125-7. ***(Invited Commentary)***.

(104) Liang, S. and **Richardson, D.R.** (2003) The effect of potent iron chelators on the regulation of p53: examination of the expression, localization and DNA-binding activity of p53 and the transactivation of WAF1. ***Carcinogenesis.*** 24(10):1601-1614.

(105) Bernhardt, P.V., Caldwell, L.M., Chaston, T.B., Chin, P. and **Richardson, D.R**. (2003) Cytotoxic iron chelators: characterization of the structure, solution chemistry and redox activity of ligands and iron complexes of the di-2-pyridyl ketone isonicotinoyl hydrazone (HPKIH) analogues. ***J Biol Inorg Chem.*** 8(8):866-80

(106) Bernhardt, P.V., Caldwell, L.M., Lovejoy, D.B. and **Richardson, D.R**. (2003) Four cytotoxic N4 substituted thiosemicarbazones derived from 2-hydroxynapthalene-1-carboxaldehyde. ***Acta Cryst. Section C*** 59, 629-633.

(107) **Richardson, D.R.** (2004) Novel chelators for central nervous system disorders that involve alterations in the metabolism of iron and other metal ions. ***Ann. N.Y. Acad. Sci.*** 1012: 326-341. ***(Invited Review).***

(108) Walcourt, A., Loyevsky, M., Lovejoy, D.B., Gordeuk, V.R. and **Richardson, D.R.** (2004) Novel aroylhydrazone and thiosemicarbazone iron chelators with anti-malarial activity against chloroquine-resistant and -sensitive parasites. ***Int. J. Biochem. Cell Biol.*** 36:401-407.

(109) Kwok, J.C. and **Richardson, D.R.** (2004) Examination of the mechanism(s) involved in doxorubicin-mediated iron accumulation in ferritin: Studies using metabolic inhibitors, protein synthesis inhibitors and lysosomotropic agents. ***Mol. Pharmacol.*** 65: 181-195.

(110) Le, N.T.V. and **Richardson, D.R.** (2004) Competing pathways of iron chelation: Angiogenesis or anti-tumor activity – targeting different intracellular iron pools to induce specific effects. ***Int. J. Cancer*** 110:468-469.

(111) **Richardson, D.R.** (2004) Mysteries of the transferrin-transferrin receptor interaction uncovered. ***Cell*** 116(4):483-485. ***(Invited Preview article)***

(112) Watts, R.N. and **Richardson, D.R.** (2004) Differential effects on cellular iron metabolism of the physiologically-relevant diatomic effector molecules, NO and CO, that bind iron. ***Biochim. Biophys. Acta – Mol. Cell. Res.*** 1692, 1-15.

(113) **Richardson, D.R.** (2004) Iron and neoplasia: The serum transferrin receptor and ferritin in prostate cancer. ***J. Lab. Clin. Med.*** 144:174-176 ***(Invited Editorial).***

(114) **Richardson, D.R.** and Morgan, E.H. (2004) The transferrin homologue, melanotransferrin, is rapidly catabolised by the liver in the mouse and does not effectively donate iron to the brain. ***Biochim. Biophys. Acta*** 1690, 124-133.

(115) Yuan J. and Lovejoy, D.B. and **Richardson, D.R.** (2004) Novel di-2-pyridyl-derived iron chelators with marked and selective anti-tumor activity: *In Vitro* and *In Vivo* Assessment. ***Blood*** 104:1450-1458

(116) Le, N.T.V. and **Richardson, D.R.** (2004) Iron chelators with high anti-proliferative activity up-regulate the expression of a growth inhibitory and metastasis suppressor gene: A novel link between iron metabolism and proliferation. ***Blood*** 104(9):2967-75.

(117) Chaston, T.B, Yuan, J., Watts, R.N. and **Richardson, D.R.** (2004) The potent anti-tumor activity of novel iron chelators derived from di-2-pyridylketone isonicotinoyl hydrazone involves Fenton-derived free radical generation ***Clin. Cancer Res.*** 10:7365-7374.

(118) Sekyere, E., Dunn, L., and **Richardson, D.R.** (2004) Expression profile of the transferrin homologue, melanotransferrin (Tumour Antigen p97), in human and mouse tissues. ***Biochim. Biophys Acta*** (in press)

(119) Bernhardt, P.V., Chin, P. and **Richardson, D.R.** (2004) Iron catalysed assembly of an asymmetric triple helicate. ***J. Chem. Soc. Dalton Trans.*** 3342-3346.

(120) Wong, C., Kwok, J.C. and **Richardson, D.R**. (2004) The novel iron chelator, 2-pyridylcarboxaldehyde isonicotinoyl hydrazone, is orally active and induces iron excretion in mice. ***Biochim. Biophys. Acta – Mol. Biol. Dis.*** **(in press)**

(121) Napier I., Ponka, P. and **Richardson, D.R.** (2004) Iron trafficking in the mitochondrion: Novel pathways revealed by disease. ***Blood*** Nov 4 [Epub ahead of print]

(122) **Richardson, D.R**. (2004) Molecular mechanisms of iron uptake by cells and the use of iron chelators for the treatment of cancer and other diseases. ***Curr. Med. Chem*** **(Invited Review; in press)**

(123) Sekyere, E., Rahmanto, Y., Dunn, L. and **Richardson, D.R**. (2004) The role of melanotransferrin in iron metabolism? **Invited Book Chapter** ***(in press).***

Invited Conference Papers

(124) Ponka, P., **Richardson, D.**, Schulman, H.M. and Baker, E. (1990) Pyridoxal isonicotinoyl hydrazone (PIH) and related acylhydrazones: Promising chelating agents for Fe overload treatment. ***Plzen Medical Report*** 62, 121-123.

(125) Lovejoy, D.B., Gao, J. and **Richardson, D.R.** (2000) Iron chelators as potential anti-neoplastic agents: Their effect on molecules involved in proliferation. Metal Ions in Biology and Medicine Volume 6, Centeno J.A., Collery, P., Vernet, G., Finkelman R.B., Gibb, H. and Etienne, J-C Eds., John Libbey Eurotext, Paris, pp221-223.

(126) Lovejoy, D.B., Bernhardt, P.V. and **Richardson, D.R.** (2000) Structure-activity relationships of novel chelators with anti-proliferative activity: The "NT" series. Metal Ions in Biology and Medicine Volume 6, Centeno J.A., Collery, P., Vernet, G., Finkelman R.B., Gibb, H. and Etienne, J-C Eds., John Libbey Eurotext, Paris, pp224-226.

(127) Watts, R.L., Wardrop, S.L. and **Richardson, D.R.** (2000) Studies on the mechanism of action of nitrogen monoxide on Iron Uptake from Transferrin. ***The Sixth Internet World Congress on Biomedical Sciences*** (see www.uclm.es/inabis2000/).

(128) Lovejoy, D.B. and **Richardson, D.R.** (2000) Structure-activity relationships of iron chelators of the pyridoxal isonicotinoyl hydrazone class. ***The Sixth Internet World Congress on Biomedical Sciences*** (see www.uclm.es/inabis2000/).

(129) Gao, J. and **Richardson, D.R.** (2000) Multiple effects of iron chelators on molecules controlling cell cycle progression. ***Transfusion Sci.*** 23:247-248.

(130) Watts, R.N. and **Richardson, D.R.** (2000) A relationship between glucose metabolism and NO-mediated iron mobilization from cells. ***Transfusion Sci.*** 23: 267-268.

(131) Gao, J., Lovejoy, D. and **Richardson, D.R.** (2000) Effect of iron chelators with potent anti-proliferative activity on the expression of molecules involved in cell cycle progression and proliferation. ***Redox Report*** 4(6):311-312.

(132) Food, M. and **Richardson, D.R.** (2002) Iron uptake by melanoma cells from the soluble form of the transferrin homologue, melanotransferrin. ***Redox Report*** 7, 279-282.

(133) Wong, C.S.M. and **Richardson, D.R.** (2004) A novel orally active chelator of the aroylhydrazone class induces iron excretion in mice. ***Biomarkers and Environment*** Suppl. 1, 1-3

(134) Nghia, L.T.V. and **Richardson, D.R.** (2004) Potent iron chelators affect multiple genes involved in the p53 tumour suppressor patheay and cell cycle control: A gene array study. ***Biomarkers and Environment*** Suppl.1, 4-6.

(135) **Richardson, D.R.** (2004) The metastasis suppressor gene, NDRG1, is up-regulated by intracellular iron depletion. ***Biomarkers and Environment (In Press).***

Articles in Preparation

(136) Watts, R.N. and **Richardson, D.R.** (2004) Multi-drug resistant protein 1 (MRP1) mediated efflux of a glutathione-NO-Fe complex. ***J. Biol. Chem.*** (in preparation).

(137) Davies, N., Chitambar CR and **Richardson, D.R.** (2004) Resistance to Gallium Results in Marked Alternations in Intracellular iron trafficking and the identification of novel intermediates involved in Fe transport. ***Blood*** (in preparation)

(138) Xu, X and **Richardson, D.R.** (2004) The molecular pharmacology of the interactions of anthracyclines with iron. ***Mol. Pharmacol.*** (in preparation).

(139) Kalinowsky, D. and **Richardson, D.R.** (2004) Evolution of iron chelators for the treatment of disease. ***Pharmacol. Rev.*** (in preparation)

(140) Kurantsin-Mills, J., Ammosova, T., Maddukuri, A., Jerebtsova, M., **Richardson, D.R.** Gordeuk V.R.,Kashanchi, F. and Nekhai, S. (2004) Dysregulation of CDK2 by iron chelators and siRNA inhibits HIV-1 transcription. (in preparation).

(141) Bernhardt, P.V., Chin, P., **Richardson, D.R.** (2004) Novel hydrazine chelators with high Fe chelation efficacy. ***J. Biol. Inorg. Chem*** (in preparation).

Patents

(142) **Richardson, D.R.**, Bernhardt, P. and Becker, E., (2000) "Iron Chelators and Uses Thereof" International Patent Application No. PCT/AU00/01050. Filing Date September 4th 2000.

(143) **Richardson, D.R**. and Lovejoy, D. (2002) Novel iron chelators as anti-tumour agents. Provisional Patent. Filed 19th March, 2002. Application: # PS1209.

(144) **Richardson, D.R.** and Lovejoy, D (2003) Aroylhydrazone derivatives and therapeutic uses thereof. Provisional Patent. Filed 5th of February, 2003. Application # 2003900495. Spruson & Ferguson ref: 624998.

(145) **Richardson, D.R.** and Lovejoy, D (2003) Aroylhydrazone derivatives and therapeutic uses thereof. Provisional Patent. Filed 5th of February, 2003. Application # 2003900496. This application is the same as the above except that it contains additional reference to the treatment of viral and parasitic diseases. Spruson & Ferguson ref: 625328.

(146) **Richardson, D.R.** and Lovejoy, D. (2003) (Thio) semicarbazone and thio (hydrazone) compounds and therapeutic uses thereof. Provisional Patent. Filed 11th of February 2003. Application # 2003900922. Spruson & Ferguson ref: 625009.

(147) **Richardson, D.R.** and Lovejoy, D. (2003) (Thio) semicarbazone and thio (hydrazone) compounds and therapeutic uses thereof. Provisional Patent. Filed 11th of February 2003. Application # 2003900600. This application

is the same as the above except that it contains additional reference to the treatment of viral and parasitic diseases. Spruson & Ferguson ref: 625514.

Abstracts

(1) Wilairat, P., Ponka, P., Vitolo, M.L., Webb, J., **Richardson, D.** and Baker, E. (1985) Effect of analogues of pyridoxal isonicotinoyl hydrazone on iron exchange with hepatocytes and macrophages. In: Proceedings of the International Conference on Thalassemia, June 30-July 3, Bangkok, Thailand, p 143.

(2) Baker, E., **Richardson, D.** and Ponka, P. (1986) Effect of analogues of PIH on iron exchange between hepatocytes and transferrin. In: Proceedings of the 21st Congress of the International Society of Hematology, Sydney, p.465.

(3) **Richardson, D.R.**, Baker, E., Vitolo, M.L., Webb, J. and Ponka, P. (1987) Evaluation of analogues of pyridoxal isonicotinoyl hydrazone for iron chelation therapy. In: Proceedings of the 26th Annual Scientific Meeting of the Australian Society for Medical Research, p. 51.

(4) Baker, E., **Richardson, D.R.**, Gross, S., Wong, A., Grady, R.W., Ponka, P., Hider, R., Kontoghiorghes, G., Peter, H. and Nozoe, S. (1987) Evaluation of new iron chelators in the hepatocyte in culture. In: Proceedings of the Eighth International Conference on Proteins of Iron Transport and Storage, Le Château Montebello, Québec, Canada, May 10-14, p. 143.

(5) **Richardson, D.R.**, Baker, E., Vitolo, L., Webb, J. and Ponka, P. (1987) Evaluation of analogues of pyridoxal isonicotinoyl hydrazone for iron chelation therapy. In: Programme guide of the Australian Society of Medical Research Young Speakers Symposium, Nurses Lecture Theatre, Queen Elizabeth II Hospital, Perth, Western Australia, December 2.

(6) **Richardson, D.R.** and Baker, E. (1988) Role of the transferrin receptor and p97 (melanotransferrin) in the iron metabolism of melanoma cells. In: Proceedings of the 27th Annual Scientific Meeting of the Australian Society for Medical Research, p. 40.

(7) **Richardson, D.R.** and Baker, E. (1989) Role of the transferrin receptor and melanotransferrin (p97) in iron uptake by human melanoma cells. In: Proceedings of the IXth International Conference on Iron Storage and Transport Proteins, Brisbane, Australia, p. 15.

(8) Baker, E., Lloyd, D., Morgan, E.H., **Richardson, D.** and Trinder, D. (1989) Mechanisms of iron uptake in normal and neoplastic cells. In: Proceedings of the IXth International Conference on Iron Storage and Transport Proteins, Brisbane, Australia, p. 108.

(9) Ponka, P., **Richardson, D.**, Schulman, H.M. and Baker, E. (1990) Pyridoxal isonicotinoyl hydrazone (PIH) and its analogues: a new group of effective iron chelating agents. In: Proceedings of the Third International Symposium on Chelating Agents in Pharmacology, Toxicology and Therapeutics, p. 33.

(10) Baker, E., **Richardson, D.**, Gross, S. and Ponka, P. (1990) Effect of hydrazones of pyridoxal, salicylaldehyde and 2-hydroxy-1-naphthylaldehyde on iron metabolism in hepatocytes in culture. In: Proceedings of the European Iron Club Meeting, Porto, 4-7 September. Arquivos de Medicina (Portugal) 5, suppl. 1: 33.

(11) **Richardson, D.R.**, Cameron, K., Turner, K.J. and Robinson, B. (1991) The mechanisms of IgE uptake by human macrophages and a B-lymphoblastoid cell line (Wil-2wt): A preliminary report. In: Proceedings of the 21st Annual Scientific Meeting of the Australian Society of Immunology, Perth, Western Australia, 4-6 December.

(12) **Richardson, D.R.**, Cikrt, M., Schulman, H.M., Baker, E. and Ponka, P. (1992) Effect of hydrazones of pyridoxal, salicylaldehyde and 2-hydroxy-1-naphthylaldehyde on iron metabolism in hepatocytes and on biliary iron excretion. In: Proceedings of the Third NIH Sponsored Symposium on the Development of Iron Chelators for Clinical Use, May 20-22, Gainesville, Florida, p. 29.

(13) **Richardson, D.R.** and Ponka, P. (1993) Examination of the ferric ammonium citrate-sensitive iron uptake process from transferrin. Identification of a redox mechanism. In: Proceedings of the IX International Conference on Iron and Iron Proteins, Jerusalem, Israel, May 2-7, p. 48.

(14) **Richardson, D.R.** (1993) The mechanisms of iron uptake from transferrin by cells in culture. In: Proceedings of the 76th Canadian Society for Chemistry Conference and Exhibition, Sherbrooke, Quebec, Canada, May 30-June 3.

(15) **Richardson, D.R.**, Neumannova, V., Nagy, E. and Ponka, P. (1993) Effect of nitric oxide species on cellular proliferation and transferrin and iron uptake by erythroleukemia (K562) cells. 35th Annual Meeting of the American Society of Hematology. ***Blood*** 82(10), Suppl. 9a.

(16) **Richardson, D.R.**, Neumannova, V. and Ponka, P. (1993) The mechanisms of iron uptake from transferrin by neuroblastoma cells and the effect of iron chelators on these processes. 35th Annual Meeting of the American Society of Hematology. ***Blood*** 82(10), Suppl. 459a.

(17) **Richardson, D.R.**, Neumannova, V., Nagy, E. and Ponka, P. (1994) Effect of nitric oxide on the iron metabolism of neoplastic cells in culture. XXV Congress of the International Society of Hematology, Cancun, Mexico. In: ***La Revista Investigacion Clinica*** Suppl. page 186.

(18) **Richardson, D.R.**, Ponka, P. and Vyoral, D. (1994) Distribution or iron in reticulocytes after inhibition of heme synthesis with succinylacetone. Examination of cytoplasmic and mitochondrial intermediates. ***Invited Poster Presentation.*** 36th Annual Meeting of the American Society of Hematology, Nashville, Tennessee, December 2-6. ***Blood*** 84(10) Suppl. 1, 112a.

(19) **Richardson, D.R.**, Tran, E.H., Bradshaw, D. and Ponka, P. (1994) Potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents. 36th Annual Meeting of the American Society of Hematology, Nashville, Tennessee, December 2-6. ***Blood*** 84(10) Suppl. 1, 613a.

(20) **Richardson, D.R.** and Baker, E. (1994) Two saturable mechanisms of iron uptake from transferrin by human melanoma cells. 36th Annual Meeting of the American Society of Hematology, Nashville, Tennessee, December 2-6. ***Blood*** 84(10) Suppl.1, 613a.

(21) **Richardson, D.R.**, Neumannova, V. and Ponka, P. (1994) Congeners of nitrogen monoxide decrease iron uptake from transferrin but do not mobilise iron from prelabelled neoplastic cells. 36th Annual Meeting of the American Society of Hematology, Nashville, Tennessee, December 2-6. ***Blood*** 84(10) Suppl. 1, 612a.

(22) **Richardson, D.R.**, Ponka, P. and Vyoral, D. (1995) The effect of the heme synthesis inhibitor, succinylacetone, on the intracellular distribution of iron in rabbit reticulocytes. In: Proceedings of the Fifth Conference of the International Association for the Study of Disorders of Iron Metabolism, Boston Marriott Copley Place, Boston, Massachusetts, April 11-13, A18, page 71.

(23) **Richardson, D.R.**, Ponka, P. and Baker, E. (1995) The effect of desferrioxamine on iron and transferrin uptake by the human melanoma cell. In: Proceedings of the Fifth Conference of the International Association for the Study of Disorders of Iron Metabolism, Boston Marriott Copley Place, Boston, Massachusetts, April 11-13, P30, page 84.

(24) Kennard, M.L., Jefferies, W.A., Gabathuler, R., Ponka, P. and **Richardson, D.R.** (1995) A novel iron uptake mechanism mediated by GPI-anchored human melanotransferrin. In: Proceedings of the International Conference on BioIron, Asheville, North Carolina, April 16-21, page 75.

(25) **Richardson, D.R.**, Neumannova, V. and Ponka, P. (1995) Redox-related species of nitrogen monoxide. Multiple effects on the iron metabolism of cancer cells. In: Proceedings of the International Conference on BioIron, Asheville, North Carolina, April 16-21, page 212.

(26) **Richardson, D.R.**, Wilczynska, A. and Baker, E. (1995) The mechanisms of iron uptake from transferrin by neoplastic cells. Evidence for two saturable processes. In: Proceedings of the International Conference on BioIron, Asheville, North Carolina, April 16-21, page 137.

(27) **Richardson, D.R.**, Ponka, P. and Vyoral, D. (1995) Intracellular iron transport intermediates in reticulocytes. Fact or fiction. In: Proceedings of the International Conference on BioIron, Asheville, North Carolina, April 16-21, page 87.

(28) **Richardson, D.R.** (1995) Multiple iron uptake pathways in neoplastic cells. Is melanotransferrin (p97) involved in iron uptake by melanoma cells ? In: Proceedings of the International Chemical Congress of the Pacific Basin Societies, Honolulu, Hawaii, December 17-22.

(29) **Richardson, D.R.** and Ponka, P. (1995) Identification of a mechanism of iron uptake by cells that is stimulated by hydroxyl radicals generated via the iron-catalyzed Haber-Weiss reaction. In: Proceedings of the 37th Annual Meeting of The American Society of Hematology, Seattle, Washington, December 1-5. ***Blood*** 86(10), Suppl. 1, 632a.

(30) **Richardson, D.R.**, Dickson, L., Ponka, P. and Baker, E. (1995) Intermediates in cellular iron uptake from transferrin. A cytoplasmic pool of iron is released from cells via temperature-dependent mechanical wounding. In: Proceedings of the 37th Annual Meeting of The American Society of Hematology, Seattle, Washington, December 1-5. ***Blood*** 86(10), Suppl. 1, 632a.

(31) **Richardson, D.R.**, Jeitner, T. and Milnes, K. (1995) Iron chelators derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone display anti-proliferative activity comparable to bleomycin. In: Proceedings of the 37th Annual Meeting of The American Society of Hematology, Seattle, Washington, December 1-5. ***Blood*** 86(10), Suppl. 1, 632a.

(32) **Richardson, D.R.** and Lheureux, F. (1995) Mobilisation of iron from prelabelled neoplastic cells by some iron chelators is an energy-dependent process. In: Proceedings of the 37th Annual Meeting of The American Society of Hematology, Seattle, Washington, December 1-5. ***Blood*** 86(10), Suppl. 1, 632a.

(33) Gabathuler, R., Kennard, M.L., Food, M., **Richardson, D.R.**, Ponka, P., Rothenberger, S. and Jefferies, W.A. (1995) Characterisation of a GPI-linked protein, melanotransferrin (p97), involved in transferrin-independent uptake of iron. FEBS 95, Basel, Switzerland, August 1995.

(34) **Richardson, D.R.**, Neumannova, V., Nagy, E. and Ponka, P. (1996) Nitrogen monoxide decreases iron uptake from transferrin but does not mobilize iron from prelabelled neoplastic cells. In: Proceedings of the International Conference on the Biochemistry and Molecular Biology of Nitric Oxide, University of California, Los Angeles, July 13-17, page 109, Abstract G4.

(35) **Richardson, D.R.**, Neumannova, V. and Ponka, P. (1996) The effect of redox-related species of nitrogen monoxide on transferrin and iron uptake and cellular proliferation of erythroleukemia (K562) cells. In: Proceedings of the The International Conference on the Biochemistry and Molecular Biology of Nitric Oxide, University of California, Los Angeles, July 13-17, Abstract G5.

(36) **Richardson, D.R.** and Ponka, P. (1997) Identification of a mechanism of iron uptake by cells that is stimulated by free radicals produced by the Haber-Weiss reaction. In: Proceedings of the International Symposium on Iron in Biology and Medicine, Saint-Malo, France, June 16-20, p. 320.

(37) **Richardson, D.R.** and Milnes, K. (1997) The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents: The mechanism of action of ligands derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone. In: Proceedings of the International Symposium on Iron in Biology and Medicine, Saint-Malo, France, June 16-20, p.165.

(38) **Richardson, D.R.** (1997) Energy-dependent mechanisms are responsible for the efflux of some iron chelators but not others. In: Proceedings of the International Symposium on Iron in Biology and Medicine, Saint-Malo, France, June 16-20, p72.

(39) **Richardson, D.R.** and Richardson, V. (1997) The effect of ferricyanide and other impermeable oxidants on the growth of human neoplastic cells. In: Proceedings of the International Symposium on Iron in Biology and Medicine, Saint-Malo, France, June 16-20, p143.

(40) **Richardson, D.R.**, Kennard, M.L., Gabathuler, R., Ponka, P. and Jefferies, W.A. (1997) Identification of a novel process of iron uptake mediated by human melanotransferrin. In: Programme of the 1997 AMP-ASMR Queensland Biomedical Research Awards, 3rd June, Brisbane, Queensland.

(41) **Richardson, D.R.** and Darnell, G. (1998). Examination of the process of iron release from cells. In: Proceedings of the Symposium in BioMedicine, Asian Pacific Association for the Study of the Liver, 11th Biennial Scientific Meeting, 16-20th February, Burswood International Resort, Perth, Western Australia, p31.

(42) **Richardson, D.R.**, Cowley, L., Anderson, G.J., Wardrop, S., and Darnell, G. (1998) The effect of nitrogen monoxide on transferrin and the RNA-binding activity of the iron-regulatory protein (IRP). In: Proceedings of the Symposium in BioMedicine, Asian Pacific Association for the Study of the Liver, 11th Biennial Scientific Meeting, 16-20th February, Burswood International Resort, Perth, Western Australia, p37.

(43) **Richardson, D.R.**, Chua, A, and Baker, E. (1998) Activation of an iron transport mechanism in hepatocytes by preincubation with low molecular weight iron complexes: Implications for the Pathogenesis of Iron Overload Disease. In: Proceedings of the Symposium in BioMedicine, Asian Pacific Association for the Study of the Liver, 11th Biennial Scientific Meeting, 16-20th February, Burswood International Resort, Perth, Western Australia, p22.

(44) Becker, E. and **Richardson, D.R.** (1998) Development of new iron chelators for the treatment of iron-loading diseases: Aroylhydrazone ligands that permeate the mitochondrion. In: Proceedings of the International Meeting on Friedreich's Ataxia, Women's and Children's Hospital Theatre, Adelaide, South Australia, August 30.

(45) **Richardson, D.R.** (1998) Mitochondrial iron transport and mitochondrial iron overload: The lessons learnt from developing erythroid cells. In: Proceedings of the International Meeting on Friedreich's Ataxia, Women's and Children's Hospital Lecture Theatre, Adelaide, South Australia, August 30.

(46) Appleyard, M., Cowley, L., Leggett, B.A., **Richardson, D.R.** and Anderson, G. (1998) Expression of the hemochromatosis gene product (HFE) and the transferrin receptor in the small intestine. In: Proceedings of the Australian Gastroenterology Conference, Canberra, Australia, October 20-23rd.

(47) Wardrop, S. and **Richardson, D.R.** (1999) The effect of intracellular iron concentration and nitrogen monoxide on Nramp2 expression and non-transferrin-bound iron uptake. In: Proceedings of the Bioiron Conference, Sorrento, Italy, May 23-28.

(48) Darnell, G. and **Richardson, D.R.** (1999) The effect of a novel iron chelator on molecular targets involved in celllular proliferation: The possible roles of WAF1 and GADD45 in cell cycle arrest. In: Proceedings of the Bioiron Conference, Sorrento, Italy, May 23-28.

(49) Becker, E., and **Richardson, D.R.** (1999) Development of novel aroylhydrazone iron chelators for the treatment of iron-loading diseases. In: Proceedings of the Bioiron Conference, Sorrento, Italy, May 23-28.

(50) **Richardson, D.R.** (1999) The effect of ceruloplasmin and other agents on iron uptake and release by HepG2 cells. In: Proceedings of the Bioiron Conference, Sorrento, Italy, May 23-28.

(51) **Richardson, D.R.** (1999) The role of melanotransferrin (tumor antigen p97) in iron uptake by the human melanoma cell. In: Proceedings of the Bioiron Conference, Sorrento, Italy, May 23-28.

(52) Wardrop, S., Watts, R. and **Richardson, D.R.** (1999) Nitrogen monoxide (NO) activates IRP-RNA binding by two possible mechanisms – a direct effect on the Fe-S cluster and iron release from cells. In: Proceedings of the Bioiron Conference, Sorrento, Italy, May 23-28.

(53) Chua, A.C.G., **Richardson, D.R.** and Baker, E. (1999) The effect of ferric ammonium citrate on iron uptake from transferrin and citrate by hepatocytes In: Proceedings of the Bioiron Conference, Sorrento, Italy, May 23-28.

(54) **Richardson, D.R.** and Bernhardt, P.V. (1999) Crystal and molecular structure of 2-hydroxy-1-napthylaldehyde isonicotinoyl hydrazone (NIH) and its iron(III) complex: An iron chelator with pronounced anti-tumour activity. Bioiron Conference, Sorrento, Italy, May 23-28.

(55) Wardrop, S.L. and **Richardson, D.R.** (1999) The iron transporter Nramp2 is not regulated like the transferrin receptor in LMTK$^-$ fibroblasts: The roles of non-iron responsive element (IRE) and the IRE-containing *Nramp2* mRNA transcripts. Combined Conference of the Australian Society for Biochemistry and Molecular Biology, Australian and New Zealand Society for Cell & Developmental Biology and Australian Society of Plant Physiologists, Conrad Jupiter's, Gold Coast, Queensland, 27-30th September.

(56) Wardrop, S.L. and **Richardson, D.R.** (1999) Interferon-γ and lipopolysaccharide differentially regulate the expression of the iron transporter Nramp2 in macrophages. Combined Conference of the Australian Society for Biochemistry and Molecular Biology, Australian and New Zealand Society for Cell & Developmental Biology and Australian Society of Plant Physiologists, Conrad Jupiter's, Gold Coast, Queensland, 27-30th September.

(57) Wardrop, S.L., Watts, R. N and **Richardson, D.R.** (1999) The mechanisms involved in nitrogen monoxide (NO) mediated iron-regulatory protein 1 (IRP1) activation and cellular iron mobilization. Combined Conference of the Australian Society for Biochemistry and Molecular Biology, Australian and New Zealand Society for Cell & Developmental Biology and Australian Society of Plant Physiologists, Conrad Jupiter's, Gold Coast, Queensland, 27-30th September.

(58) Watts, R.N. and **Richardson, D.R.** (1999) Nitrogen monoxide (NO) and glucose: Unexpected links that affect intracellular iron metabolism and iron mobilisation. In: Proceedings of the Australian Physiological and Pharmacological Society 30(2):40P. Newcastle, New South Wales, Australia, 27-29th September.

(59) Lovejoy, D., Bernhardt, P.V., and **Richardson, D.R.** (1999) Novel "hybrid" iron chelators derived from aroylhydrazones and thiosemicarbazones demonstrate marked anti-proliferative activity against tumour cells. In: Proceedings of the Australian Physiological and Pharmacological Society 30(2):113P. Newcastle, New South Wales, Australia, 27-29th September.

(60) **Richardson, D.R.** (1999) Iron and gallium salts increase iron uptake from transferrin by human melanoma cells. Further investigation of the ferric ammonium citrate-stimulated iron uptake process. In: Proceedings of the Australian Physiological and Pharmacological Society 30(2):102P. Newcastle, New South Wales, Australia, 27-29th September.

(61) Watts, R.N. and **Richardson, D.R.** (1999) Glucose unexpectantly modulates the effect of nitrogen monoxide on intracellular iron metabolism: The possible role of redox-related processes in the access of NO to intracellular iron pools ? Proceedings of the International Conference on Oxidative Pathways in Health and Disease, Veterinary Faculty Conference Centre, University of Sydney, Sydney, 1-4th of December.

(62) Wardrop, S.L. and **Richardson, D.R.** (1999) Lipopolysaccharide and/or interferon-γ regulate the expression of Nramp2, Nramp1 and the transferrin receptor independently of the iron-regulatory protein in macrophages: A possible role of Nramp2 in host defence ? Proceedings of the International Conference on Oxidative Pathways in Health and Disease, Veterinary Faculty Conference Centre, University of Sydney, Sydney, 1-4th of December.

(63) Lovejoy, D.B., Bernhardt, P., and **Richardson, D.R.** (1999) Identification of novel chelators for the treatment of cancer: The thiosemicarbazone-aroylhydrazone mix. Proceedings of the International Conference on Oxidative Pathways in Health and Disease, Veterinary Faculty Conference Centre, University of Sydney, Sydney, 1-4th of December.

(64) Becker E., Watts, R.N., Lovejoy, D. and **Richardson, D.R.** (1999) Three classes of novel aroylhydrazone iron chelators and their structure-activity relationships. Proceedings of the International Conference on Oxidative Pathways in Health and Disease, Veterinary Faculty Conference Centre, University of Sydney, Sydney, 1-4th of December.

(65) Gao, J., Lovejoy, D. and **Richardson, D.R.** (1999) Effect of iron chelators with potent anti-proliferative activity on the expression of molecules involved in cell cycle progression and proliferation. Proceedings of the International Conference on Oxidative Pathways in Health and Disease, Veterinary Faculty Conference Centre, University of Sydney, Sydney, 1-4th of December.

(66) **Richardson, D.R.** (1999) Melanotransferrin (p97) does not play a significant role in iron uptake by the human

melanoma cell line SK-MEL-28. Proceedings of the International Conference on Oxidative Pathways in Health and Disease, Veterinary Faculty Conference Centre, University of Sydney, Sydney, 1-4th of December.

(67) Lovejoy, D. and **Richardson, D.R.** (2000) Novel hydrazone ligands show high anti-proliferative activity: The 2-hydroxy-1-naphthylaldehyde thiosemicarbazone analogues. Proceedings of the 10th International Conference on Oral Chelators, Cyprus, 22-26th of March, p26.

(68) Gao, J. and **Richardson, D.R.** (2000) Multiple effects of iron chelators on molecules controlling cell cycle progression. Proceedings of the 10th International Conference on Oral Chelators, Cyprus, 22-26th of March, p30.

(69) Watts, R.N. and **Richardson, D.R.** (2000) A relationship between glucose metabolism and NO-mediated iron mobilization from cells. Proceedings of the 10th International Conference on Oral Chelators, Cyprus, 22-26th of March, p32.

(70) **Richardson, D.R.**, Lovejoy, D. and Gao, J. (2000) Structure-activity relationships reveal that anti-proliferative activity can be separated from iron chelation efficacy: Aroylhydrazone chelators can be designed for the potential treatment of cancer and iron overload disease. The Second International Conference on HIV and Iron, Brugge, Belgium, March 31st-April 1st. ***J. Clin. Virol.*** 16:100-101.

(71) Lovejoy, D.B. and **Richardson, D.R.** (2000) Condensation of 2-hydroxy-1-naphthylaldehyde with a range of thiosemicarbazides yields chelators with high anti-proliferative activity. The Second International Conference on HIV and Iron, Brugge, Belgium, March 31st-April 1st. ***J. Clin. Virol.*** 16:101.

(72) Watts, R.N. and **Richardson, D.R.** (2000) Nitrogen monoxide mediated iron efflux: The roles of glucose, glutathione and intracellular redox state. The Second International Conference on HIV and Iron, Brugge, Belgium, March 31st-April 1st. ***J. Clin. Virol.*** 16:78-79.

(73) Gao, J., Lovejoy, D., Becker, E. and **Richardson, D.R.** (2000) The effect of an iron chelator with potent antiproliferative effects on the expression and activity of molecules involved in proliferation and cell cycle control: A comparison between desferrioxamine (DFO) and 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone (311). The Second International Conference on HIV and Iron, Brugge, Belgium, March 31st-April 1st. ***J. Clin. Virol.*** 16:102.

(74) Wardrop, S.L. and **Richardson, D.R.** (2000) Regulation of macrophage iron metabolism by lipopolysaccharide and interferon-γ: The effect on iron uptake and the expression of Nramp2, Nramp1 and the transferrin receptor. The Second International Conference on HIV and Iron, Brugge, Belgium, March 31st-April 1st. ***J. Clin. Virol.*** 16:101-102.

(75) Lovejoy, D.B., Gao, J. and **Richardson, D.R.** (2000) Iron chelators as potential anti-neoplastic agents: Their effect on molecules involved in proliferation. Proceedings of the 6th International Symposium on Metal Ions in Biology and Medicine, "Metals in Oncology Session", 7 - 10th May, San Juan, Puerto Rico, USA, p107.

(76) Lovejoy, D.B., Bernhardt, P., and **Richardson, D.R.** (2000) Structure-activity relationships of novel chelators with anti-proliferative activity: The NT series. Proceedings of the 6th International Symposium on Metal Ions in Biology and Medicine, "Metals in Oncology Session", 7 - 10th May, San Juan, Puerto Rico, USA, p318.

(77) Lovejoy, D. and **Richardson, D.R.** (2000) Novel iron chelators show high anti-cancer activity. Proceedings of the Australian Society of Medical Research (NSW) Meeting, Veterinary Conference Centre, The University of Sydney, June 5.

(78) Watts, R. and **Richardson, D.R.** (2000) Nitric oxide, glucose and iron release from cells: Parting is such sweet sorrow. Proceedings of the Australian Society of Medical Research (NSW) Meeting, Veterinary Conference Centre, The University of Sydney, June 5.

(79) **Richardson, D.R.** and Ponka, P. (2000) Iron Metabolism: Conference Summary. Conference Proceedings of the Sixth Internet World Congress for Biomedical Sciences, Internet Association of Biomedical Sciences, Garcia-Rojo, M. Ed., Ciudad Real, Spain, p 15-16.

(80) Gao, J. and **Richardson, D.R.** (2001) The role of iron in G1/S progression: Iron chelators affect the expression of critical regulatory molecules involved in cell cycle control. Proceedings of the ASMR (NSW) Scientific Meeting, The Scientia, University of New South Wales, June 4th.

(81) Le, N., **Richardson, D.R.**, Watts, R. N., Lovejoy, D. and Chaston, T. (2001) The Potent Ribonucleotide Reductase Inhibitor, 3-Aminopyridine-2-Carboxaldehyde Thiosemicarbazone, Acts as a Chelator and Forms a Redox-Active Iron Complex. Proceedings of the ASMR (NSW) Scientific Meeting, The Scientia, University of New South Wales, June 4th.

(82) Watts, R.N. and **Richardson, D.R.** (2001) The cytotoxic effector molecule, nitric oxide, acts as a potent iron chelator to mobilize iron from cells. Proceedings of the ASMR (NSW) Scientific Meeting, The Scientia, University of New South Wales, June 4th.

(83) Kwok, J. and **Richardson, D.R**. (2001) The potent anti-tumour agents, anthracyclines, affect crucial regulatory molecules involves in iron metabolism. Proceedings of the ASMR (NSW) Scientific Meeting, The Scientia, University of New South Wales, June 4th.

(84) Lovejoy, D. and **Richardson, D.R.** (2001) Iron chelators with anti-tumour activity: Structure activity relationships and mechanisms of action. Proceedings of the ASMR (NSW) Scientific Meeting, The Scientia, University of New South Wales, June 4th.

(85) Chaston, T.C. and **Richardson, D.R.** (2001) 2-Pyridyl-Carboxaldehyde isonicotinoyl hydrazone analogues: iron chelators that prevent iron-mediated free radical generation, implications for the treatment of iron overload disease. Proceedings of the ASMR (NSW) Scientific Meeting, The Scientia, University of New South Wales, June 4th.

(86) Sekyere, E. and **Richardson, D.R.** (2001) Determination of the expression profile of the mouse melanotransferrin gene. Proceedings of the ASMR (NSW) Scientific Meeting, The Scientia, University of New South Wales, June 4th.

(87) **Richardson, D.R.**, Chin, P., and Bernhardt, P.V. (2001) Unprecedented oxidation of biologically active hydrazone chelator catalysed by iron(III): Serendipitous identification of diacylhydrazone ligands with high iron chelation efficacy. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(88) Watts, R.N. and **Richardson, D.R.** (2001) Mechanisms involved in the nitric oxide-mediated mobilisation of iron from cells. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(89) Kwok, J.C. and **Richardson, D.R.** (2001) The effect of doxorubicin and its analogues on the iron metabolism of neoplastic cells: Doxorubicin decreases total iron-regulatory protein 1 levels. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(90) Becker, E., Greer, J. and **Richardson, D.R.** (2001) Frataxin expression is regulated by erythroid differentiation and protoporphyrin IX: Clues to a role in heme and iron metabolism of mammalian cells. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(91) Gao, J. and **Richardson, D.R.** (2001) The role of iron in cell cycle control: Potent iron chelators affect multiple targets involved in cell cycle progression. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(92) Lovejoy, D. and **Richardson, D.R.** (2001) Novel hybrid iron chelators show high anti-tumour activity. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(93) Becker, E., Lovejoy, D., Watts, R. and **Richardson, D.R.** (2001) Iron chelators with multiple roles: The development and evaluation of compounds for the treatment of iron overload disease and cancer. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(94) Chaston, T.C. and **Richardson, D.R.** (2001) 2-pyridyl-carboxaldehyde isonicotinoyl hydrazone analogues:

Potential iron chelators for the treatment of iron overload disease that prevent iron-mediated free radical generation. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(95) **Richardson, D.R.**, Mouralian, C., Ponka, P. and Becker E. (2001) Lipophilic chelators that bind mitochondrial iron: Potential agents for the treatment of Friedreich's ataxia. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(96) Green, D.A., Antholine, W.E., **Richardson, D.R**. and Chitambar, C.R. (2001) Ribonucleotide reductase as a preferential target for the inhibition of leukemic cell growth by 311, a novel iron chelator of the pyridoxal isonicotinoyl hydrazone class. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(97) Bernhardt, P.V., Chin, P., and **Richardson, D.R.** (2001) Unprecedented oxidation of a biologically active aroylhydrazone chelator catalysed by iron(III): Serendipitous identification of diacylhydrazone ligands with high iron chelation efficacy. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(98) Chua, A.C.G., **Richardson, D.R.**, Savigni, D.L., Baker, E. (2001) The potential of novel aroylhydrazones in iron chelation therapy: Studies using the rat hepatocyte model. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(99) Sekyere, E. and **Richardson, D.R.** (2001) The expression profile of the mouse melanotransferrin (p97) gene. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(100) Chaston, T., Lovejoy, D., Le, N., Watts, R. N., and **Richardson, D.R.** (2001) Triapine acts as an iron chelator and forms a redox-active iron complex: Selective anti-proliferative activity against neoplastic cells occurs by a different mechanism to that found for DFO and 311. Proceedings of the Bioiron 2001 World Congress on Iron Metabolism, Cairns, August 18-23, Australia.

(101) Gao, J. and **Richardson, D.R.** (2001) Iron and cell cycle progression. The VIth Conference of the International Society for Trace Element Research in Humans, Quebec City, Quebec, CANADA, September 15-20.

(102) Becker, E., Greer, J. and **Richardson, D.R.** (2001) The role of frataxin in mitochondrial iron metabolism. The VIth Conference of the International Society for Trace Element Research in Humans, Quebec City, Quebec, CANADA, September 15-20.

(103) Kwok, J. and **Richardson, D.R**. (2001) Anthracyclines modify the RNA-binding activity of iron-regulatory proteins. The VIth Conference of the International Society for Trace Element Research in Humans, Quebec City, Quebec, CANADA, September 15-20.

(104) Lovejoy, D. and **Richardson, D.R.** (2001) Hybrid chelators derived from thiosemicarbazones and aroylhydrazones show marked anti-cancer activity. The VIth Conference of the International Society for Trace Element Research in Humans, Quebec City, Quebec, CANADA, September 15-20.

(105) Watts, R.N. and **Richardson, D.R.** (2001) NO mediates cellular iron mobilization. The VIth Conference of the International Society for Trace Element Research in Humans, Quebec City, Quebec, CANADA, September 15-20.

(106) **Richardson, D.R.**, Mouralian, C., Ponka, P. and Becker E. (2001) Development of iron chelators of the PCIH class for the treatment of Friedreich's ataxia: Chelators that bind mitochondrial iron. The VIth Conference of the International Society for Trace Element Research in Humans, Quebec City, Quebec, CANADA, September 15-20.

(107) Gao, J. and **Richardson, D.R.** (2001) Iron and cellular proliferation: The use of potent iron chelators as anti-tumor agents. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(108) Kwok, J. and **Richardson, D.R**. (2001) Anthracyclines and their Metal Ion Complexes Affect the RNA-Binding Activity of the Iron-Regulatory Protein 1. Proceedings of the International Conference on Redox Processes in

Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(109) Lovejoy, D. and **Richardson, D.R.** (2001) Novel "Hybrid" Iron Chelators Derived from Aroylhydrazones and Thiosemicarbazones Demonstrate High Anti-Proliferative Activity that is Selective for Tumor Cells. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(110) Becker, E., Greer, J. and **Richardson, D.R.** (2001) Frataxin is down-regulated by erythroid differentiation and protoporphyrin IX: Evidence for a role in mitochondrial Fe sequestration for haem synthesis. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(111) Dena A. Green, William E. Antholine, **Des R. Richardson,** and Christopher R. Chitambar (2001) Ribonucleotide Reductase as a Target for Inhibition of Leukemic Cell Growth by 311, a Novel Iron Chelator of the Pyridoxal Isonicotinoyl Hydrazone Class. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(112) Chaston, T.B. and **Richardson, D.R.** (2001) The development of the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone class of chelators as therapeutic agents for the treatment of iron overload disease: Redox activity of the iron complexes. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(113) Watts, R.N. and **Richardson, D.R.** (2001) Novel links between the metabolism of glucose and the mobilization of iron from cells by nitric oxide: The role of glutathione and the identification of ferritin as a major intracellular target. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(114) Food, M. and **Richardson, D.R.** (2001) The soluble form of the membrane-bound transferrin homologue, melanotransferrin, does not donate iron to cells via the transferrin-binding site. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(115) Chaston, T., Lovejoy, D., Watts, R.N., **Richardson, D.R.**, (2001) Examination of the Anti-Proliferative Activity of Iron Chelators: Multiple Cellular Targets and the Different Mechanism of Action of Triapine compared to Desferrioxamine and the Potent PIH Analogue 311. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(116) Sekyere, E. and **Richardson, D.R.** (2001) Expression of the transferrin homologue, melanotransferrin (tumour antigen p97) in the mouse. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(117) Erika M. Becker, David B. Lovejoy, Ralph Watts, and **Des R. Richardson.** (2001) Novel Aroylhydrazone Iron Chelators Differ in their Iron Chelation Efficacy and Anti-Proliferative Activity: Identification of a New Class of Potential Anti-Proliferative Agents. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(118) Simon Liang and **Des R. Richardson** (2001) Iron Chelation Increases $p21^{WAF/CIP1}$ mRNA Levels in Cells with Mutant p53: The Possible Roles of the Transcription Factors AP2, Sp1, and Sp3 in p21 Expression. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(119) Nghia Le and **Des R. Richardson** (2001) The Effect of Potent Iron Chelators on Cell Cycle Control Molecules in Normal and Neoplastic Cells: Translation of p21/WAF1 is Dependent on Intracellular Iron Levels. Proceedings of

the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001 .

(120) **Des R. Richardson**, Cindy Mouralian, Prem Ponka, and Erika Becker (2001) Chelators of the PCIH Class Demonstrate Potential for the Treatment of Friedreich's Ataxia : Ligands that Deplete Mitochondrial Iron. Proceedings of the International Conference on Redox Processes in Chemistry, Biology and Medicine, Veterinary Faculty Conference Centre, University of Sydney, Sydney, Nov 30-Dec 4th 2001.

(121) Chaston, T.B. and **Richardson, D.R.** (2002) The redox activity of the iron complexes of novel chelators for the treatment of iron overload. In: Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(122) Chaston, T.B, Lovejoy, D.B., Watts, R.N. and **Richardson, D.R.** (2002) Multiple cellular targets and different mechanisms of action of iron chelators with anti-proliferative activity. In: Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(123) Le, N.T.V. and **Richardson, D.R.** (2002) Translation of the potent cell cycle inhibitor $p21^{WAF1/CIP1}$ is dependent on intracellular iron levels. In: Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(124) Liang, S. and **Richardson, D.R.** (2002) Iron chelation increases $p21^{WAF1/CIP1}$ mRNA levels in cells with mutant p53: The possible roles of AP2, SP1 and SP3. In: Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(125) Lovejoy, D.B. and **Richardson, D.R.** (2002) Novel "hybrid" hydrazone iron chelators demonstrate selective anti-proliferative activity against tumor cells. In: Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(126) Becker, E.M., Lovejoy, D.B., Greer, J.M., Watts, R.N. and **Richardson, D.R.** (2002) The di-pyridyl ketone isonicotinoyl hydrazones: Potent iron chelators and inhibitors of DNA synthesis. In: Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(127) Food, M.R., Sekyere, E.O. and **Richardson, D.R.** (2002) A soluble form of the transferrin homologue, melanotransferrin, donates iron to cells. In: Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(128) Kwok, J.C. and **Richardson, D.R.** (2002) Unexpected anthracycline-mediated alterations in iron regulatory protein RNA-binding activity in tumor cells. In : Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(129) Watts, R.N. and **Richardson, D.R.** (2002) Nitric oxide intercepts iron before uptake into ferritin and mobilizes iron in a glutathione-dependent manner. In : Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(130) Becker, E.M., Greer, J.M., Ponka, P. and **Richardson, D.R.** (2002) Role of frataxin in iron metabolism: Erythroid differentiation down-regulates frataxin expression. In: Proceedings of the Conference on Advances in Trace Elements, Minerals and Vitamins in Humans: Functional and Clinical Aspects, April 10-13, 2002, Monastir, Tunisia.

(131) Kwok, J. and **Richardson, D.R.** (2002) Unexpected effects of anthracyclines on iron regulatory protein 1 and 2: The anthracycline-iron and anthracycline-copper complexes decrease RNA-binding activity. In: Proceedings of the European Iron Club, Porto, Portugal, September 25-28.

(132) Kwok, J.C. and **Richardson, D.R.** (2002) Anthracyclines inhibit the iron mobilization pathway from ferritin in cardiomyocytes and neoplastic cells: Inhibition of the ferritin mobilization pathway. In: Proceedings of the European Iron Club, Porto, Portugal, September 25-28.

(133) Le, N.T.V and **Richardson, D.R.** (2002) Nuclear translocation of the universal cyclin-dependent kinase inhibitor, $p21^{WAF1/CIP1}$ is prevented by potent iron chelators. In: Proceedings of the European Iron Club, Porto, Portugal, September 25-28.

(134) Food, M.R., Sekyere, E.O. and **Richardson, D.R.** (2002) A soluble form of the membrane-bound transferrin homologue, melanotransferrin (tumour antigen p97), inefficiently donates iron to cells. In: Proceedings of the European Iron Club, Porto, Portugal, September 25-28.

(135) Lovejoy, D. and **Richardson, D.R.** (2002) Novel PIH analogue iron chelators demonstrate selective anti-proliferative activity against tumor cells. In: Proceedings of the European Iron Club, Porto, Portugal, September 25-28.

(136) Watts, R.N. and **Richardson, D.R.** (2002) Nitric oxide inhibits iron uptake into ferritin and indirectly increases iron mobilization from the protein. In: Proceedings of the European Iron Club, Porto, Portugal, September 25-28.

(137) Chaston, T.B. and **Richardson, D.R.** (2002) The 2-pyridyl-carboxaldehyde isonicotinoyl hydrazone class of chelators and their Interactions with Iron and DNA: Implications for Toxicity in the Treatment of Iron Overload Disease. In: Proceedings of the European Iron Club, Porto, Portugal, September 25-28.

(138) Chaston, T.C. and **Richardson, D.R.** (2002) Interactions of the 2-pyridyl-carboxaldehyde isonicotinoyl hydrazone class of chelators with iron and DNA: Implications for toxicity in the treatment of iron overload disease. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(139) Kwok, J.C. and **Richardson, D.R.** (2002) Anthracycline-Fe and -Cu complexes unexpectedly decrease iron regulatory protein-RNA binding activity. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(140) Kwok, J.C. and **Richardson, D.R.** (2002) Anthracyclines inhibit the intracellular mobilization of iron from ferritin in myocardial and neoplastic cells. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(141) Le, N.T.V. and **Richardson, D.R.** (2002) Transport of the cyclin-dependent kinase inhibitor, $p21^{WAF1/CIP1}$ to the nucleus is prevented by potent iron chelators. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(142) Becker, E.M., Lovejoy, D.B., Greer, J.M., Watts, R.N, and **Richardson, D.R.** (2002) Identification of the Dipyridyl Ketone Isonicotinoyl Hydrazones: Potent Iron Chelators and Inhibitors of DNA Synthesis. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(143) Becker, E.M., Lovejoy, D.B., Greer, J.M., Watts, R.N, and **Richardson, D.R.** (2002) Role of frataxin in iron metabolism: Erythroid differentiation down-regulates frataxin expression. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(144) Watts, R.N.and **Richardson, D.R.** (2002) Nitric oxide intercepts iron before incorporation into ferritin and indirectly mobilizes iron from the protein in a glutathione-dependent manner. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(145) Kwok, J.C. and **Richardson, D.R.** (2002) The Iron Mobilization Pathway from Ferritin is Inhibited in Normal and Neoplastic Cells. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(146) Food, M.R., Sekyere, E.O. and **Richardson, D.R.** (2002) Soluble Melanotransferrin Ineffectively Donates Iron

to Cells. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(147) Chaston, T.B., Lovejoy, D.B., Watts, R.N. and **Richardson, D.R.** (2002) Multiple Cellular Targets and Different Mechanisms of Action of Iron Chelators with Anti-Proliferative Activity. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(148) Liang, S. and **Richardson, D.R.** (2002) Iron chelation increases $p21^{WAF/CIP1}$ mRNA levels in cells with mutant p53: The possible roles of AP2, SP1 and SP3. Second Conference on the Molecular Mechanisms of Metal Toxicity and Carcinogenesis, National Institute for Occupational Safety and Health, Morgantown, West Virginia, September 8-11.

(149) Walcourt, A, Loyevsky M, Lovejoy, D., Gordeuk, V.R. and **Richardson, D.R.** (2002) Anti-malarial activity of new iron chelators of the aroylhydrazone class. International Meeting of the American Society for Tropical Medicine and Hygiene, November 10-14, Denver, Colorado, USA.

(150) Watts, R.N. and **Richardson, D.R.** (2002) The effects of nitric oxide on cellular iron metabolism. In: Proceedings of The Australian Health and Medical Research Congress. Melbourne Convention Centre, November 25-29.

(151) Sekyere, E., Food, M.R. and **Richardson, D.R.** (2002) Expression profile of the transferrin homologue, melanotransferrin (tumour antigen p97), and studies on its thermolysin-like metalloproteinase activity. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania.

(152) Le, N.T.V. and **Richardson, D.R.** (2002) Intracellular iron levels regulate the nuclear translocation of the universal cell cycle inhibitor $p21^{WAF1/CIP1}$. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania.

(153) Chaston T.B., Lovejoy, D., Watts, R.N. and **Richardson, D.R.** (2002) Iron chelators inhibit neoplastic cell proliferation by different mechanisms of action. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania.

(154) S.X. Liang and **Richardson, D.R.** (2002) Iron chelation increases $p21^{WAF/CIP1}$ mRNA levels in cells with mutant p53: The possible roles of AP2, SP1, and SP3. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania.

(155) Lovejoy, D.B. and **Richardson, D.R.** (2002) Novel anti-metabolites for tumour therapy: Iron chelators with high anti-proliferative activity. In: Proceedings of the Mutagenesis and Experimental Pathology Society of Australia, December 4-6, Hobart, Tasmania.

(156) **Richardson, D.R.** (2002) The use of iron chelators for the treatment of cancer, Friedreich's ataxia and iron overload disease. In: Proceedings of the Australian Society of Anaesthesia Technicians, 6th Annual Educational Conference and Scientific Exhibition, "Development Beyond Technology", 14-16th of November, Sydney

(157) Le, N.T.V. and **Richardson, D.R**. (2002) Nuclear translocation of the universal cell cycle inhibitor $p21^{WAF1/CIP1}$ is dependent on intracellular iron. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(158) Liang, S.X. and **Richardson, D.R.** (2002) The effect of the potent cytotoxic iron chelator, 311, on the regulation of $p21^{WAF1/CIP1}$ mRNA expression: A p53-independent pathway. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(159) Watts, R.N. and **Richardson, D.R.** (2002) Nitrogen monoxide and carbon monoxide: mirror-image effector molecules that bind iron but have different effects on cellular iron metabolism. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(160) Food, M., Sekyere, E. and **Richardson, D.R.** (2002) The soluble form of melanotransferrin inefficiently donates iron to cells via non-specific internalization and degradation of the protein. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(161) Kwok, J. and **Richardson, D.R**. (2002) Possible role of superoxide in the inhibition of the iron mobilization pathway from ferritin by anthracyclines. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(162) Chaston, T.B. and **Richardson, D.R.** (2002) Interactions of the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone analogues with iron and DNA: Implications for the treatment of iron overload disease. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(163) Wong, C. and **Richardson, D.R.** (2002) The novel iron chelator, PCTH, is orally active and induces iron excretion from mice that is comparable to deferiprone and pyridoxal isonicotinoyl hydrazone. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(164) Lovejoy, D.B. and **Richardson, D.R.** (2002) In vivo effects of iron chelators with high anti-proliferative activity. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(165) Sekyere, E., Food, M.R. and **Richardson, D.R.** (2002) Melanotransferrin does not possess thermolysin-like metalloproteinase activity and its tissue expression profile is different to that of the transferrin receptor 1. In: Proceedings of the Oxidative Pathways in Chemistry, Biology and Medicine Conference, Wollongong, Wollongong University, December 14-16.

(166) Becker, E., Greer, J., Ponka, P. and **Richardson, D.R.** (2003) Erythroid differentiation and protoporphyrin IX down-regulate frataxin expression: Characterisation of frataxin expression compared to molecules involved in iron metabolism and hemoglobinisation. Friedreich's Ataxia Research Conference, National Institutes of Health, Bethesda, Maryland, USA. February 14-16.

(167) **Richardson, D.R.**, Mouralian, C., Ponka, P. and Becker, E. (2003) Development of iron chelators of the PCIH class for the treatment of Friedreich's ataxia: Chelators that bind mitochondrial iron. Friedreich's Ataxia Research Conference, National Institutes of Health, Bethesda, Maryland, USA. February 14-16

(168) Chaston, T.B. and **Richardson, D.R.** (2003) Iron chelators of the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone class as potential agents to treat Friedreich's ataxia. Friedreich's Ataxia Research Conference, National Institutes of Health, Bethesda, Maryland, USA. February 14-16.

(169) Watts, R.N., Baranano, D.E., Synder, S.H. and **Richardson, D.R**. (2003) Carbon monoxide (CO) and nitric oxide (NO) and their effects on cellular iron trafficking: Are CO and NO mirror-image effector molecules ? Proceedings of the ASMR 12th NSW Scientific Meeting, Powerhouse Museum, 2nd June, Sydney, NSW.

(170) Dunn, L.L., Sekyere, E.O., Food, M.R. and **Richardson, D.R.** (2003) Expression of the malignant melanoma tumour antigen, p97 (melanotransferrin), in mice and humans. Proceedings of the ASMR 12th NSW Scientific Meeting, Powerhouse Museum, 2nd June, Sydney, NSW.

(171) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines and lysosomal inhibitors induce iron accumulation in the iron storage protein ferritin. Proceedings of the ASMR 12th NSW Scientific Meeting, Powerhouse Museum, 2nd June, Sydney, NSW.

(172) Wong, C. and **Richardson, D.R**. (2003) The novel iron chelator, PCTH, is orally active and induces iron excretion from mice that is comparable to deferiprone and pyridoxal isonicotinoyl hydrazone. Proceedings of the ASMR 12th NSW Scientific Meeting, Powerhouse Museum, 2nd June, Sydney, NSW.

(173) Yuan, J., Lovejoy, D.B. and **Richardson, D.R.** (2003) Investigations of the anti-tumour effects and apoptotic pathways induced by iron chelators in vitro and in vivo. Proceedings of the ASMR 12th NSW Scientific Meeting, Powerhouse Museum, 2nd June, Sydney, NSW.

(174) Chaston, T.B. and **Richardson, D.R.** (2003) The novel di-2-pyridyl ketone isonicotinoyl hydrazone class of cytotoxic iron chelators promote Fenton chemistry and cause DNA damage *in vitro*. Proceedings of the ASMR 12th NSW Scientific Meeting, Powerhouse Museum, 2nd June, Sydney, NSW.

(175) Liang, S.X. and **Richardson, D.R.** (2003) The effect of iron chelators on p53: Iron chelation increases WAF1 mRNA expression by a p53-independent pathway. Proceedings of the ASMR 12th NSW Scientific Meeting, Powerhouse Museum, 2nd June, Sydney, NSW.

(176) Le, N.T.V and **Richardson, D.R.** (2003) Potent iron chelators affect multiple genes involved in the p53 tumour suppressor pathway and cell cycle control: A gene array study. Proceedings of the ASMR 12th NSW Scientific Meeting, Powerhouse Museum, 2nd June, Sydney, NSW.

(177) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines and lysosomal inhibitors induce iron accumulation in the iron storage protein ferritin by preventing iron mobilization from the molecule. In Proceedings of the 13th International Conference on Oral Chelation and Therapy, July 10-15th , Prague, Czech Republic.

(178) Yuan, J., Lovejoy, D.B. and **Richardson, D.R.** (2003) Investigations of the anti-tumour effects and apoptotic pathways induced by iron chelators in vitro and in vivo. In Proceedings of the 13th International Conference on Oral Chelation and Therapy, July 13-15th , Prague, Czech Republic.

(179) Liang, S.X. and **Richardson, D.R.** (2003) The effect of iron chelators on p53: Iron chelation increases WAF1 mRNA expression by a p53-independent pathway. In Proceedings of the 13th International Conference on Oral Chelation and Therapy, July 13-15th , Prague, Czech Republic.

(180) Le, N.T.V and **Richardson, D.R.** (2003) Potent iron chelators affect multiple genes involved in the p53 tumour suppressor pathway and cell cycle control: A gene array study. In: Proceedings of the 13th International Conference on Oral Chelation and Therapy, July 13-15th, Prague, Czech Republic.

(181) Le, N.T.V and **Richardson, D.R.** (2003) Potent iron chelators increase the mRNA levels of the universal cyclin-dependent kinase inhibitor, $p21^{CIP1/WAF1}$, but paradoxically inhibit its translation: A potential mechanism of cell cycle dysregulation. In: Proceedings of the 13th International Conference on Oral Chelation and Therapy, July 13-15th , Prague, Czech Republic.

(182) Wong, C. and **Richardson, D.R**. (2003) The novel iron chelator, PCTH, is orally active and induces iron excretion from mice that is comparable to deferiprone and pyridoxal isonicotinoyl hydrazone. In: Proceedings of the 13th International Conference on Oral Chelation and Therapy, July 10-15th , Prague, Czech Republic.

(183) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines and lysosomal inhibitors induce iron accumulation in the iron storage protein ferritin. Proceedings of the European Iron Club, Vienna, Austria, August 20-23.

(184) Liang, S.X. and **Richardson, D.R.** (2003) The effect of potent iron chelators on p53: Characterisation of their effects on p53 expression, localisation, DNA-binding activity and transactivation of WAF1. Proceedings of the European Iron Club, Vienna, Austria, August 20-23.

(185) Sekyere, O., Dunn, L.L., Food, M.R. and **Richardson, D.R**. (2003) Expression profile of the transferrin homologue, melanotransferrin (tumour antigen p97), and studies on its thermolysin-like metalloproteinase activity. Proceedings of the European Iron Club, Vienna, Austria, August 20-23.

(186) Watts, R.N. and **Richardson, D.R.** (2003) A possible pathway of nitric oxide cytotoxicity through the removal of iron from cells. Proceedings of the European Iron Club, Vienna, Austria, August 20-23.

(187) Watts, R.N., Synder, S. and **Richardson, D.R.** (2003) Nitrogen monoxide and carbon monoxide: Another journey through the looking glass. Proceedings of the European Iron Club, Vienna, Austria, August 20-23.

(188) Bernhardt, P.V., Chin, P. and **Richardson, D.R.** (2003) Orally active chelators for the treatment of iron overload disorders. Proceedings of the 11th International Conference on Biological Inorganic Chemistry (ICBIC) held in the Cairns Convention Centre, Cairns, Australia 19-23 July 2003.

(189) Richardson, D.R., Le N.T.V and Bernhardt, P.V. (2003) Development of iron chelators as effective anti-tumour agents: Examination of their effects on molecules involved in cell cycle control and proliferation. Proceedings of the National ASMR conference, Adelaide, South Australia, Nov. 23-25th.

(190) Castelnoble, L.A. and **Richardson, D.R.** (2003) The role of N-myc in the regulation of iron metabolism in neuroblastoma cells. National ASMR Conference, Adelaide, South Australia, Nov. 23-25th.

(191) Dunn, L.L., Sekyere, E. and **Richardson, D.R**. (2003) Expression of melanotransferrin (melanoma tumour antigen p97) in the human and the mouse. National ASMR Conference, Adelaide, South Australia, Nov. 23-25th

(192) Napier, I.A., Xie, L. and **Richardson, D.R.** (2003) Examination of the role of frataxin in mitochondrial iron metabolism. National ASMR conference, Adelaide, South Australia, Nov. 23-25th

(193) Kwok, J.C. and **Richardson, D.R.** (2003) National ASMR conference, Adelaide, South Australia, Nov. 23-25th

(194) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines inhibit ferritin iron mobilization: A novel additional mechanism of cytotoxicity. Tow Prize Meeting, Sydney, NSW, Nov. 7

(195) Nurtjahja-Tjendraputra, E., Le, N.T.V. and **Richardson, D.R.** (2003) Potent iron chelators affect the expression of multiple molecules involved in cell cycle progression, metastasis, DNA excision, and apoptosis. Proceedings of the Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28.

(196) Wong, C.S.M. and **Richardson, D.R.** (2003) The acute and subchronic effect of PCTH on iron excretion in mice. Proceedings of the Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28.

(197) Le, N.T.V. and **Richardson, D.R.** (2003) The metastasis suppressor gene, *NDRG1*, is markely up-regulated by iron chelators but not the DNA-damaging agent, actinomycin D. Proceedings of the Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28.

(198) Xie, L., Napier, I.A. and **Richardson, D.R.** (2003) Examination of the pathophysiological function of frataxin using cells transfected with an anti-sense frataxin expression vector. Proceedings of the Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28.

(199) Castelnoble, L.A., Le, N.T.V., and **Richardson, DR.** (2003) Iron chelators overcome N-MYC repression of NDRG1 in neuroblastoma cells. Proceedings of the Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28.

(200) Dunn. L.L., Sekyere, E.O. and **Richardson, D.R.** (2003) The melanoma tumour antigen p97 (Melanotransferrin): Expression studies and splice variants in humans and mice. Proceedings of the Fifth Annual Mutagenesis Experimental Pathology Society of Australia Conference, Sydney, Nov 26-28.

(201) Wong, C.S.M. and **Richardson, D.R.** (2003) The induction of iron excretion by PCTH, a novel ORally Active Chelator, Acutely and Subchronically in Mice. Proceedings of the International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, Dec. 4-7.

(202) Richardson, D.R. and Le, N.T.V (2003) Molecular mechanisms involved in the regulation of cell cycle progression by iron: The development of novel iron chelators as potent anti-proliferative agents. Proceedings of the International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, Dec. 4-7.

(203) Nurtjahja-Tjendraputra, E., Le, N.T.V. and **Richardson, D.R.** (2003) Effects of potent iron chelators on the

expression of molecules involved in cell cycle progression and proliferation. Proceedings of the International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, Dec. 4-7.

(204) Kwok, J.C. and **Richardson, D.R.** (2003) Anthracyclines inhibit the mobilization of iron from ferritin: A potential additional mechanism of cytotoxicity that could involve the lysosome and proteasome. Proceedings of the International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, Dec. 4-7.

(205) Watts, R.N. and **Richardson, D.R.** (2003) NO-Mediated Iron Release is Dependent on Glutathione and is Transported from Cells by the GSH-Conjugate Transporter, MRP1. Proceedings of the International Joint Meeting on Food Factors and Free Radicals in Health and Disease, Kyoto, Japan, Dec. 4-7.

(206) Le, N.T.V. and **Richardson, D.R.** (2004) p53-independent induction of the metastasis suppressor gene NDRG1 by potent iron chelators. Sixteenth Lorne Cancer Conference, Lorne, Victoria, 12-15th Feb.

(207) Castelnoble, L.A., Le, N.T.V. and **Richardson, D.R.** (2004) Effect of iron chelators on the expression of N-myc and its target gene, NDRG1, in neuroblastoma cells. Sixteenth Lorne Cancer Conference, Lorne, Victoria, 12-15th Feb.

(208) Sekyere, E., Dunn, L.L. and **Richardson DR.** (2004) Expression studies of the tumour antigen, p97, melanotransferrin. 25th Lorne Genome Conference, Lorne, Victoria, 15-19th February.

(209) Castelnoble, L.A., Le, N.T.V and **Richardson, D.R.** (2004) Iron chelators overcome N-myc repression of Ndrg1 in neuroblastoma cells. Fourteenth International Conference on Oral Chelation, Utrecht, The Netherlands, April 14-17th.

(210) Le, N.T.V. and **Richardson, D.R.** (2004) Potent iron chelators but not the DNA-damaging agent actinomycin D markedly up-regulate the metastasis suppressor gene, Ndrg1. Fourteenth International Conference on Oral Chelation, Utrecht, The Netherlands, April 14-17th.

(211) Yuan, J., Lovejoy, D.B. and **Richardson, D.R.** (2004) Novel Di-2-Pyridyl-Derived Iron Chelators with Marked and Selective Anti-Tumor Activity: *In Vitro* and *In Vivo* Assessment. European Society for Clinical Investigation and Fourteenth International Conference on Oral Chelation, Utrecht, The Netherlands, April 14-17th.

(212) Watts, R.N. and **Richardson, D.R.** (2004) The Mobilization of an NO-Fe Complex from Cells is Mediated by Active Transport – The Possible Role of the Glutathione Conjugate Transporter, MRP1. European Society for Clinical Investigation, Utrecht, The Netherlands, April 14-17th.

(213) Watts, R.N. and **Richardson, D.R.** (2004) A cellular paradox: Does the detoxifying protein MRP1 assist in NO-mediated cytotoxicity? Society of Free Radical Research International XII Biennial Meeting, Crowne Plaza Panamricano, Buenos Aires, Argentina, May 5-9.

(214) Le, N.T.V. and **Richardson, D.R.** (2004) Pharmacological induction of the metastasis suppressor gene, *Ndrg-1*, in cancer cells: A potential therapeutic mechanism. Australian Society of Medical Research NSW Conference, Sydney, June 7.

(215) Dunn, L.L., Sekyere, E.O. and **Richardson, D.R.** (2004) Elucidating the function of the melanoma tumour antigen p97 (melanotransferrin). Australian Society of Medical Research NSW Conference, Sydney, June 7.

(216) Nurtjahja-Tjendraputra, E., Le, N.T.V. and **Richardson, D.R.** (2004) Iron chelators: Novel anti-tumour agents that induce cyclin D degradation and affect the expression of molecules involved in cell cycle progression, DNA excision and apoptosis. Australian Society of Medical Research NSW Conference, Sydney, June 7.

(217) **Richardson, D.R.** and Le, N.T.V. (2004) Dissection of novel molecular mechanisms involved in inhibiting cancer cell proliferation and cell cycle progression by iron depletion. Proceedings of the 7th International Symposium on Chelating Agents in Biomedicine, Toxicology and Therapeutics held in Pilsen, Czech Republic on July 8 -11, 2004.

(218) **Richardson, D.R.** and Le, N.T.V. (2004) The role of iron in cell cycle progression and the use of iron chelators

as anti-tumour agents. Australian Health and Medical Research Congress, Symposium on Biometals-Mutagenesis and Experimental Pathology Society of Australia Conference, Sydney, November 23-25.

(219) **Richardson, D.R.** and Le, N.T.V. (2004) Expression of the metastasis and growth suppressor gene, *NDRG1*, is regulated by intracellular iron via HIF-1α-dependent and -independent mechanisms. Proceedings of the European Iron Club, Rennes, France, September 8-11.

(220) **Richardson, D.R.**, Yuan, J. and Lovejoy, D. (2004) Novel DpT Fe chelators show marked and selective anti-tumour activity in vitro and in vivo. Proceedings of the European Iron Club, Rennes, France, September 8-11.

(221) Davies, N., Chitambar, C. and **Richardson, D.R.** (2004) Resistance to gallium nitrate in human leukaemia cells is associated with marked alterations in iron trafficking pathways. Proceedings of the European Iron Club, Rennes, France, September 8-11.

(222) **Richardson, D.R.** and Nurtjahja, E. (2004) Iron chelators: Novel anti-tumour agents that inhibit cell cycle progression and induce decreased cyclin D1 and P21 expression. Proceedings of the European Iron Club, Rennes, France, September 8-11.

(223) Dunn, L.L. and **Richardson, D.R.** (2004) Inhibition of melanotransferrin (p97) expression in melanoma cells inhibits proliferation and cellular migration. Proceedings of the European Iron Club, Rennes, France, September 8-11.

(224) Park, J. and **Richardson, D.R.** (2004) Effects of frataxin hyper-expression on intracellular iron trafficking. Proceedings of the European Iron Club, Rennes, France, September 8-11.

(225) Caldwell, L.M. Bernhardt, P.V., Chaston, T.B., Chin, P. and **Richardson, D.R.** (2004) Cytotoxic iron chelators: Iron complexes of di-2-pyridyl ketone isonicotinoyl hydrazone (HPKIH) analogues. Proceedings of the 36th International Inorganic Chemistry Conference, Merida, Mexico, July.

(226) Whitnall, M., Howard, J. and **Richardson, D.R.** (2004) Novel anti-tumour agents show rapid and broad activity against a range of cancer cells and overcome resistance to clinically used cytotoxics. Hunter Medical Research Institute "New Therapeutics" Conference, Newcastle, 4th-6th October.

(227) Le, N. and **Richardson, D.R.** (2004) Unexpected links between iron metabolism and the expression of the metastasis suppressor gene, *Ndrg1*: Iron depletion using chelators up-regulates *Ndrg1* expression. Second Australian Health and Medical Research Congress, November 21-26.

(228) Dunn, L.L. and **Richardson, D.R.** (2004) Inhibition of melanotransferrin (p97) expression in melanoma cells inhibits proliferation and cell migration. Second Australian Health and Medical Research Congress, November 21-26.

(229) Davies, N., Chitambar, C.R. and **Richardson, D.R.** (2004) Resistance to gallium nitrate in human leukaemia cells is associated with marked alterations in iron trafficking pathways. Second Australian Health and Medical Research Congress, November 21-26.

(230) Whitnall, M., Howard, J. and **Richardson, D.R.** (2004) Novel anti-tumour agents show rapid and broad activity against a range of cancer cells and overcome resistance to clinically used cytotoxics. Second Australian Health and Medical Research Congress, November 21-26.

(231) Dunn, L.L., Yahmato, Y., Sekyere, E.O. and **Richardson, D.R.** (2004) Elucidating the function of the melanoma tumour antigen p97 (melanotransferrin). Oxidants and Anti-Oxidants in Chemistry and Biology. The Thirteenth Annual Conference of the Society of Free Radical Research Australasia, Christchurch, New Zealand, 3-5 Dec.

(232) Xu, X. and **Richardson DR**. (2004) Anthracycline mediated ferritin-Fe accumulation is not protective against cytotoxicity induced by iron-loading. Oxidants and Anti-Oxidants in Chemistry and Biology. The Thirteenth Annual Conference of the Society of Free Radical Research Australasia, Christchurch, New Zealand, 3-5 Dec.

(233) Watts RN and **Richardson DR** (2004) A Radical Transporter and a New Model of NO Release?: The

Detoxifying Protein MRP1 Assists in Nitric Oxide (NO)-Mediated Fe Export from Cells. The Thirteenth Annual Conference of the Society of Free Radical Research Australasia, Christchurch, New Zealand, 3-5 Dec.

SECTION F – REFEREES

1. Professor Roger Dean Ph.D., D.Sc (Former Director)
Executive Director, The Heart Research Institute (until Jan. 4th 2002)
From Feb 1st 2002:
Vice Chancellor, University of Canberra
Vice-Chancellor's Office
University of Canberra
Canberra ACT 2601
ph: 02 6201 5000
email: roger.dean@canberra.edu.au

2. Associate Professor Erica Baker (Honours, MSc. and Ph.D supervisor)
Department of Physiology
University of Western Australia
Nedlands, WA 6907
Australia
Ph: +61-8-9380-3932
FAX: +61-8-9380-1025
Email: baker@cyllene.uwa.edu.au

3. Professor Ulf T. Brunk
Professor of Pathology and Chairman
Division of Pathology II
Linkoping Universitet
S-581 85 Linkoping
Sweden
Ph: +46-13-221-515
FAX: +46-13-221-529
Email: ulf.brunk@pat.liu.se

4. Professor Christopher Chitambar
Division of Hematology/Oncology
Medical College of Wisconsin
9200 W. Wisconsin Ave
Milwaukee, WI 53226, USA
Ph: +1-414-805-4604
FAX: +1-414-805-4606
Email: chitambr@mcw.edu

5. Professor Daniel Vyoral
Institute of Hematology and Blood Transfusion
U Nemocnice 1, Praha 2
Prague, 12000 Czech Republic
FAX: 420-2-219-77-370
Email: vyoral@uhkt.cz

IN THE UNITED STATES PATENTS AND TRADEMARK OFFICE

Applicant:	**The Heart Research Institute Ltd. and The University of Queensland**
Serial No:	**10/069923**
Filed:	**4 September 2000**
Title:	**Iron chelators and uses thereof**
Inventors:	**Des Richardson *et al.***

ANNEXURE B

This is Annexure B referred to in the Statutory Declaration of Des Richardson made before me this fifteenth day of December 2004.

R L Butler

ARTICLE IN PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochimica et Biophysica Acta xx (2004) xxx–xxx

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

http://www.elsevier.com/locate/bba

PCTH: a novel orally active chelator of the aroylhydrazone class that induces iron excretion from mice

C.S.M. Wong, J.C. Kwok, D.R. Richardson*

Children's Cancer Institute Australia for Medical Research, Iron Metabolism and Chelation Program, PO Box 81, High St, Randwick, Sydney, New South Wales, 2031, Australia

Heart Research Institute, 145 Missenden Rd, Camperdown, Sydney, New South Wales, 2050, Australia

Received 24 June 2004; received in revised form 29 August 2004; accepted 1 September 2004

Abstract

β-Thalassaemia major is an inherited blood disorder which is complicated by repeated blood transfusion and excessive gastrointestinal iron (Fe) absorption, which leads to toxic Fe overload. Current treatment using the chelator, desferrioxamine (DFO), is expensive and cumbersome since the drug requires long subcutaneous infusions and it is not orally active. A novel chelator, 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone (PCTH), was recently designed and shown to have high Fe chelation efficacy in vitro [E.M. Becker, D.R. Richardson, J. Lab. Clin. Med. 134 (1999) 510–521; D.R. Richardson, et al., Biochim. Biophys. Acta 1536 (2001) 133–140]. The aim of this investigation was to examine the Fe chelation efficacy of PCTH in vitro implementing primary cultures of cardiomyocytes and in vivo using mice. We showed that PCTH was significantly ($P<0.005$) more effective than DFO at mobilising ^{59}Fe from prelabelled cardiomyocytes. Moreover, PCTH prevented the incorporation of ^{59}Fe into ferritin during Fe uptake from ^{59}Fe-labelled transferrin. These effects were important to assess as cardiac complications caused by Fe deposition are a major cause of death in β-thalassaemia major patients. Further studies showed that PCTH was orally active and well tolerated by mice at doses ranging from 50 to 200 mg/kg, twice daily (*bd*), for 2 days. A dose-dependent increase in faecal ^{59}Fe excretion was observed in the PCTH-treated group. This level of Fe excretion at 200 mg/kg was similar to the same dose of the orally effective chelators, pyridoxal isonicotinoyl hydrazone (PIH) and deferiprone (L1). Effective Fe chelation in the liver by PCTH was shown via its ability to reduce ferritin-^{59}Fe accumulation. Mice treated for 3 weeks with PCTH at doses of 50 and 100 mg/kg/*bd* showed no overt signs of toxicity as determined by weight loss and a range of biochemical and haematological indices. In subchronic Fe excretion studies over 3 weeks, PIH and PCTH at 75 mg/kg/*bd* for 5 days/week increased faecal ^{59}Fe excretion to 140% and 145% of the vehicle control, respectively. This study showed that PCTH was well tolerated at 100 mg/kg/*bd* and induced considerable Fe excretion by the oral route, suggesting its potential as a candidate to replace DFO.

Keywords: Iron; Iron chelator; Pyridoxal isonicotinoyl hydrazone

1. Introduction

Iron (Fe)-loading diseases such as β-thalassaemia are treated using chelation therapy [1–3]. The most common treatment utilises desferrioxamine (DFO; Fig. 1), the only Fe chelator widely approved for clinical use [2]. Unfortunately, DFO is orally ineffective because it is poorly absorbed across the gastrointestinal tract [2]. Therefore, to effect appreciable Fe excretion, a cumbersome regimen of subcutaneous infusion of DFO, 12–24 h/day, 5–6 days/week is required [1–3]. Additionally, a third

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DFO, desferrioxamine; FA, Friedreich's ataxia; L1, deferiprone; PCBH, 2-pyridylcarboxaldehyde benzoyl hydrazone; PCBBH, 2-pyridylcarboxaldehyde *m*-bromobenzoyl hydrazone; PCIH, 2-pyridylcarboxaldehyde isonicotinoyl hydrazone; PCTH, 2-pyridylcarboxaldehyde 2-thiophenecarboxylhydrazone; PIH, pyridoxal isonicotinoyl hydrazone; Tf, transferrin

* Corresponding author. Iron Metabolism and Chelation Program, Children's Cancer Institute Australia for Medical Research, High St, PO Box 81, Randwick, Sydney, New South Wales, 2031, Australia. Tel.: +61 2 9382 0046; fax: +61 2 9382 0060.

E-mail address: d.richardson@ccia.org.au (D.R. Richardson).

0925-4439/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.bbadis.2004.09.001

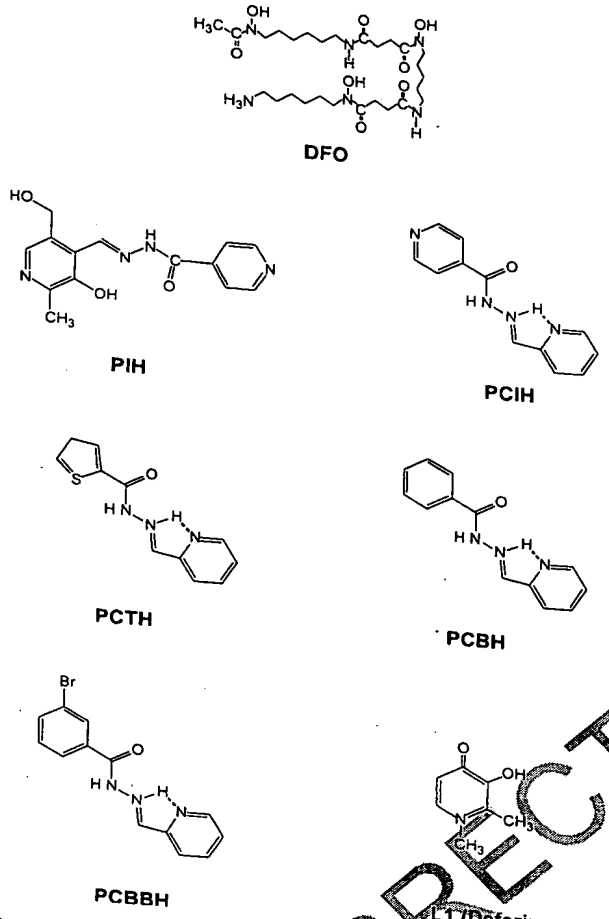


Fig. 1. The chemical structures of the chelators examined in this investigation, namely: desferrioxamine (DFO), pyridoxal isonicotinoyl hydrazone (PIH), 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH), 2-pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone (PCTH), 2-pyridylcarboxaldehyde benzoyl hydrazone (PCBH), 2-pyridylcarboxaldehyde *m*-bromobenzoyl hydrazone (PCBBH) and L1 (deferiprone).

of the patients receiving DFO experience pain and swelling at the site of injection, resulting in low compliance [1–3]. These difficulties associated with DFO administration have created much interest in designing Fe chelators that are orally effective, inexpensive and simple to synthesise [3].

Currently, the only orally effective chelator used as a substitute for DFO in some countries is 3-hydroxy-1,2-dimethylpyrid-4-one (L1; also known as DMHP, Deferiprone, or Ferriprox®; Fig. 1) [4–8]. The use of this drug is controversial [4], as there have been inconsistencies reported regarding its safety and efficacy [4,9,10]. Currently, L1 is approved for clinical use in Europe but not the United States of America nor most other Western countries [8]. More recent reports suggest that the combination of DFO and L1 leads to greater Fe chelation efficacy and lower toxicity than L1 alone, and could be useful for the treatment of β-thalassaemic patients [4,11–13]. Unfortunately, this regimen does not overcome a major problem of DFO therapy, that is, the need for long subcutaneous infusions [1–3].

Another chelator with oral activity is pyridoxal isonicotinoyl hydrazone (PIH; Fig. 1) [14–18]. This tridentate ligand shows many properties of an ideal Fe chelator for clinical use, as it is: (1) orally active, (2) permeates cell membranes easily, (3) shows high selectivity for Fe(III) over other metal ions, and (4) is simple to synthesise [18]. Moreover, a small clinical trial with PIH showed considerable activity, despite the relatively low doses that were given [19]. However, the lack of patent protection on PIH diminished pharmaceutical interest in this compound, prompting the design and patenting of a novel group of chelators, the 2-pyridylcarboxaldehyde isonicotinoyl hydrazone (PCIH) analogues (Fig. 1) [20–22].

Our laboratory has previously shown that some of the PCIH analogues have high efficiency at mobilising Fe from various cell types in culture [20,21]. The release of Fe by these PCIH analogues was at least twofold higher than that achieved by DFO, and notably, some of these ligands were more active than PIH at low concentrations [20]. These novel chelators were also more effective than DFO at preventing Fe uptake from transferrin (Tf) in vitro [20]. The PCIH analogues exhibited low toxicity and anti-proliferative activity in vitro which is important for long-term chelation therapy in patients [20,23]. The potential of the PCIH analogues as treatment for Fe-loading diseases has expanded to include Friedreich's ataxia (FA), which is a severe neurodegenerative disease that results in mitochondrial Fe-loading [24–27]. The Fe-loading is due to a mutation in the FRDA gene that encodes the molecule, frataxin, which is involved in mitochondrial Fe metabolism [26–30]. Decreased levels of frataxin in FA patients and the deposition of Fe in this highly redox-active organelle result in oxidative stress [31,32], which plays a role in the pathogenesis of the disease [27]. Interestingly, the PCIH analogues were shown to be effective at permeating the mitochondrion and mobilising a stored Fe pool, providing a potential therapeutic strategy for FA [21]. Furthermore, like PIH, the PCIH analogues are easily and economically synthesised by a one-step Schiff base condensation, overcoming the high cost of DFO [18,20,33,34].

The aim of the current investigation was to determine the efficacy of pyridylcarboxaldehyde 2-thiophenecarboxyl hydrazone (PCTH) and other PCIH analogues at Fe chelation in vitro in primary cardiomyocyte cultures. This was important, as the heart is a site that becomes loaded with Fe in β-thalassaemia major and this can lead to a fatal cardiomyopathy [2,5,12,11]. The marked activity of PCTH in vitro then led to studies in vivo in mice. These studies demonstrated that PCTH was orally effective, showing Fe chelation efficacy comparable to the orally active chelators, PIH and L1.

2. Materials and methods

2.1. Cell treatments and reagents

The hydrochloride salts of PIH and the PCIH analogues were synthesised and characterised by standard techniques [33, 34]. We purchased DFO from Novartis Pharmaceuticals (Basel, Switzerland). All other chemicals were of analytical reagent quality.

2.2. Cell culture

Primary cultures of neonatal myocardial cells were isolated from 2–3-day-old rats using well-established methods [35–40]. Briefly, ventricles were minced and incubated in the presence of 0.05% collagenase type II (Worthington, Lakewood, NJ, USA) at 37 °C. The cell suspension was then centrifuged in a Percoll gradient (1.05 g/mL; Amersham Biosciences, Uppsala, Sweden) to purify cardiomyocytes from other cell types including fibroblasts and red blood cells [38,40]. Cells were plated on collagen-coated plates and cultured at 37 °C in a humidified atmosphere of 8% O_2/5% CO_2 [38,40]. Experiments were performed on day 4 of culture. Purity of cardiomyocyte cultures was confirmed by immunofluorescent staining of the cells using an α-actinin antibody (Sigma Chemical Co., St. Louis, MO, USA) [39,40]. Neonatal rat cardiomyocytes were used in our studies as they demonstrate many of the functional characteristics of the intact heart [35]. Further, the effects of Fe chelators on these cells have been well characterised and this model has been shown to closely mimic the in vivo situation [35–37,40].

2.3. Preparation of ^{59}Fe-transferrin and ^{59}Fe-lactoferrin

Rat apoTf (kindly provided by Professor E.H. Morgan, Department of Physiology, University of Western Australia, Perth, Australia) and human lactoferrin (Sigma) were labelled with ^{59}Fe (Dupont-NEN, MA, USA) to produce $^{59}Fe_2$-transferrin (^{59}Fe-Tf) or $^{59}Fe_2$-lactoferrin, respectively, as previously described [41,42]. Unbound ^{59}Fe was removed by exhaustive vacuum dialysis against 0.15 M NaCl buffered to pH 7.4 using 1.4% $NaHCO_3$. Fully saturated diferric Tf and diferric lactoferrin were used in all experiments.

2.4. Effect of chelators on ^{59}Fe efflux from prelabelled cardiomyocytes

Iron efflux experiments examining the ability of various chelators to mobilise ^{59}Fe from cells were performed using established techniques [43]. Briefly, cells were prelabelled with ^{59}Fe-Tf ([Tf]=0.75 μM; [Fe]=1.5 μM) for 18 h at 37 °C. This medium was aspirated and the cell monolayer washed six times with ice-cold Hank's balanced salt solution (HBSS; Invitrogen, Mount Waverley, Australia). The cells were then reincubated for 24 h at 37 °C with minimum essential medium with Earle's salts (MEM; Invitrogen) in the presence or absence of the chelators to be tested. After this incubation, the overlying media containing released ^{59}Fe was collected in γ-counting tubes. The cells were removed from the petri dishes using a plastic spatula and placed in a separate set of tubes. Radioactivity was measured in both the cell pellet and supernatant using a γ-scintillation counter (Wallac Wizard 3, Turku, Finland).

2.5. Animals

Male, 6-week-old Balb/c mice (University of Adelaide, Animal Services, South Australia) were used in all studies. Prior to the commencement of experiments, the mice were divided randomly into groups of three for the acute studies and groups of 10 for the toxicology studies. The animals were housed in filtered-top cages unless specified, with food and water given *ad libitum*. Mice were exposed to an alternating 12-h period of light and dark. This study was approved by the Central Sydney Area Health Service Animal Welfare Committee (Royal Prince Alfred Hospital Zone) and the University of New South Wales Animal Care and Ethics Committee. All experimental work was carried out in registered animal facilities.

2.6. Acute and subchronic administration of PCTH: ^{59}Fe excretion studies

Mice were randomly divided into groups of three and injected via the tail-vein with ^{59}Fe (New England Nuclear, MA, USA) bound to lactoferrin at 6 μCi/mg [44]. The animals were then placed in metabolic cages that separately collect urine and faeces (Techniplast, Buguggiate, Italy). We used ^{59}Fe-lactoferrin in our studies as it has been described to result in hepatocyte ^{59}Fe-labelling in mice [44]. Faecal and urine samples were collected daily throughout the experimental time course and the amount of ^{59}Fe measured using the gamma counter described above. Twenty eight hours after the ^{59}Fe injection, mice were given by gavage either the vehicle control (20% propylene glycol in 0.9% saline) or the chelators dissolved in this vehicle at the following doses: PCTH (50–200 mg/kg), PIH (200 mg/kg) or L1 (200 mg/kg). The various treatments were given twice daily (*bd*), 6 h apart, for 2 days after which the mice were sacrificed. Livers of the mice were obtained for determining the distribution of intracellular ^{59}Fe using native PAGE ^{59}Fe-autoradiography by the method described below.

In subchronic studies, mice were labelled with ^{59}Fe-lactoferrin as above and then 28 h after the initial ^{59}Fe injection, the mice were given by gavage either the vehicle control or PIH or PCTH at a dose of 75 mg/kg/*bd* dissolved in the vehicle. The chelator was administered for 5 days/week for 3 weeks and faeces collected every 24 h. The ^{59}Fe excreted was calculated per gram of faeces produced.

2.7. Assessment of the nature of soluble ^{59}Fe-containing molecules using native-page-^{59}Fe-autoradiography

Native PAGE ^{59}Fe-autoradiography was performed using established techniques [40,43,45].

Briefly, to determine the effects of chelators on the intracellular distribution of ^{59}Fe, cardiomyocytes were incubated with ^{59}Fe-Tf (0.75 μM) for 18 h at 37 °C, washed six times, and then reincubated for 24 h at 37 °C in the presence of control medium (MEM) or medium containing 50 μM DFO or PCIH. The cells were then lysed using 30 μL of ice-cold 1.5% Triton X-100 containing 2 mM phenylmethylsulfonyl fluoride (PMSF; Sigma), followed by one freeze–thaw cycle. The samples were then vortexed and centrifuged at 14,000 rpm for 45 min at 4 °C using a refrigerate microfuge (Hettich EBA 12R, Germany). The supernatants were collected and loaded at 100 μg protein/lane and electrophoresed on a 5% native PAGE gel at 15 mA/gel for 2–3 h at 4 °C. The gel was subsequently placed onto a Hybond N$^+$ membrane (Amersham Biosciences, UK) and dried under vacuum at 80 °C for 2 h (Slab Gel Dryer 2000, Selby Biolab, Australia). The dried membrane was then exposed to X-ray film and ^{59}Fe-autoradiography performed.

In experiments examining the effects of chelators on ^{59}Fe uptake from ^{59}Fe-Tf (0.75 μM) and its subsequent incorporation into ferritin, cardiomyocytes were incubated for 18 h at 37 °C with ^{59}Fe-Tf (0.75 μM) in the presence of control medium (MEM) or medium containing 50 μM DFO or PCIH. The cells were then washed six times, lysed, and the native PAGE ^{59}Fe-autoradiography technique performed as described above.

To determine the intracellular distribution of ^{59}Fe in the liver from mice labelling experiments, a small piece of liver (~3 mm^3) was minced in 200 μL of ice-cold 1.5% Triton X-100 (Sigma) containing 2 mM PMSF, followed by one freeze–thaw cycle. All subsequent procedures were the same as that described above.

2.8. Toxicological Assessment of PCTH

Toxicological studies were conducted using 10 mice per group. The four experimental groups examined were the vehicle control (20% propylene glycol in 0.9% NaCl) and PCTH at doses of 50, 100 and 200 mg/kg, administered orally by gavage twice daily (*bd*) for 3 weeks. The mice were monitored daily for signs of distress or toxicity such as the state of the fur and eyes, lethargy, diarrhoea and tremor. Body weights and food intake were also measured weekly. After 3 weeks of treatment and just prior to sacrifice, 0.7–1 mL of blood was obtained by cardiac puncture using sodium pentobarbitone-induced anaesthesia. Blood samples from each animal were divided into two aliquots and placed in EDTA and Li-Heparin tubes for haematology and biochemistry analyses, respectively (IDEXX Laboratories, Rydalmere, Sydney, NSW, Australia). Upon sacrifice, gross necropsy and histopathology were performed on all experimental groups and assessed by an experienced histopathologist (Mayne Healthy Vetnostics, North Ryde, NSW, Australia).

2.9. Statistical analysis

Experimental data were compared using Student's *t*-test. Results were considered statistically significant when $P<0.05$. All experiments were repeated two to three times.

3. Results

3.1. The Effect of the PCIH analogues on iron mobilisation from cardiomyocytes and the intracellular distribution of iron after iron uptake from transferrin

Our previous studies examined the effect of the PCIH chelators at inducing ^{59}Fe release and preventing ^{59}Fe uptake from ^{59}Fe-Tf using a well-characterised cell line in our laboratory, namely SK-N-MC neuroepithelioma cells [20]. Indeed, these cells were previously used to screen a wide variety of Fe chelators for activity [43,46,47]. In the current study, we assessed the ability of chelators to mobilise ^{59}Fe from primary cultures of cardiomyocytes prelabelled with ^{59}Fe-Tf. These cells were vital to assess as the heart is an important site of Fe-loading and consequent pathology in β-thalassaemia major [2,5,11,12]. In addition, cardiomyocytes in culture show many properties of heart cells in vivo, including contractility, automaticity and rhythmicity [35–37].

In these experiments, cardiomyocytes were prelabelled with ^{59}Fe-Tf (0.75 μM) for 18 h at 37 °C, washed, and then reincubated for 24 h at 37 °C in the presence of DFO (50 μM) or the four most effective PCIH analogues (50 μM) identified from our previous studies (i.e., PCIH, PCTH, PCBH, and PCBBH) [20]. Cardiomyocytes released 18±2% of total cellular ^{59}Fe upon reincubation with control media only and this was increased to 33±4% and 34±1% after incubation with DFO and PCIH, respectively (Fig. 2A). The three other PCIH analogues, namely PCTH, PCBH and PCBBH, showed significantly ($P<0.005$) greater activity at increasing ^{59}Fe release than DFO and PCIH, increasing it to 51–52% of total cellular ^{59}Fe (Fig. 2A). The total amount of ^{59}Fe (i.e., released ^{59}Fe plus cellular ^{59}Fe) was not significantly different ($p>0.05$) under all experimental conditions (Fig. 2B). Hence, the differential ^{59}Fe release found in the presence of the chelators could not be explained by different total ^{59}Fe levels. These experiments confirmed the higher Fe chelation activity of PCTH, PCBH and PCBBH compared to DFO and PCIH that was previously demonstrated using SK-N-MC cells [20]. Considering the results above, and our previous studies [20], the chelator with greatest Fe chelation efficacy and lowest antiproliferative activity was PCTH. Hence, this ligand was

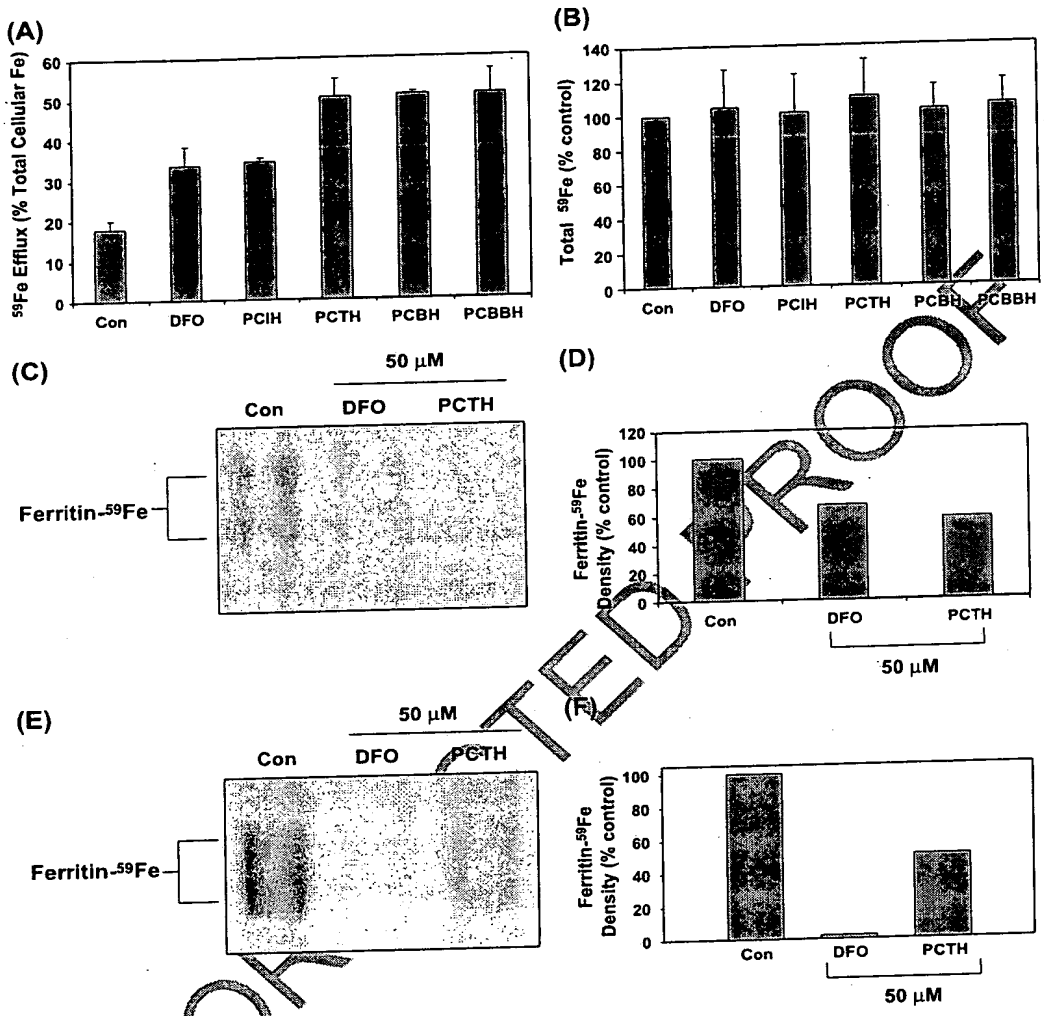


Fig. 2. (A) The PCIH analogues, PCTH, PCBH and PCBBH, were more effective than DFO at inducing ^{59}Fe efflux from primary rat cardiomyocyte cultures. (B) The total amount of ^{59}Fe (i.e., efflux and cells) is equal under all experimental conditions. For both (A) and (B), primary cultures of rat cardiomyocytes were prelabelled with ^{59}Fe-transferrin (^{59}Fe-Tf, 0.75 μM) for 18 h at 37 °C, washed, and then reincubated for 24 h at 37 °C and ^{59}Fe released from the cells into the medium was then assessed (see Materials and methods). Results are the means±S.D. of three separate experiments. (C) The chelator, PCTH (50 μM), showed similar activity as DFO (50 μM) at decreasing ferritin-^{59}Fe levels of prelabelled cardiomyocytes. Primary cultures of cardiomyocytes were prelabelled with ^{59}Fe-Tf (0.75 μM) for 18 h at 37 °C. The cells were washed and reincubated for 24 h at 37 °C with either control medium or medium containing DFO (50 μM) or PCTH (50 μM). The cells were then lysed and native PAGE ^{59}Fe-autoradiography performed (see Materials and methods). (D) Densitometric analysis of the results in (C). The results in (C) and (D) are typical from three separate experiments. (E) The chelators, DFO and PCTH, decrease ^{59}Fe uptake into ferritin of cardiomyocytes. Cardiomyocytes were incubated with ^{59}Fe-Tf (0.75 μM) for 18 h at 37 °C in the presence of either control medium, DFO (50 μM), or PCIH (50 μM). (F) Densitometric analysis of the results in (E). The results in (E) and (F) are a typical from three separate experiments.

chosen to be further evaluated in more detail in vitro and in vivo studies.

The ability of PCTH compared to DFO at mobilising ^{59}Fe from the Fe storage molecule, ferritin, in cardiomyocytes was assessed using native PAGE ^{59}Fe-autoradiography (Fig. 2C and D). In these studies, the cells were labelled for 18 h at 37 °C with ^{59}Fe-Tf (0.75 μM), washed and then reincubated with medium alone (control) or medium containing DFO (50 μM) or PCTH (50 μM). Uptake of ^{59}Fe into control cardiomyocytes was mainly incorporated into ferritin which ran as two diffuse partially overlapping bands on native PAGE gels (Fig. 2C), as we showed previously [40]. Confirmation that these two bands were ferritin was confirmed by supershift analysis using an anti-ferritin antibody (Roche Diagnostics, NY, USA) [40]. These broad bands are quite different to that found in other cell types where ferritin runs as a single intense ^{59}Fe-containing band [40,43,45]. This broad ferritin band in cardiomyocytes is consistent with multiple ferritin species. Previous studies have documented a glycosylated ferritin from heart tissue

that is smaller than cellular ferritin and is also cross-reactive with serum ferritin [48]. Additionally, cleavage of ferritin within siderosomes has been documented, giving rise to different mobilities of the molecule on native PAGE [49]. The reincubation of labelled cardiomyocytes with DFO and PCTH reduced ferritin-^{59}Fe levels to 50% and 45%, respectively, of that found with cells incubated with control medium only (Fig. 2C and D). The ability of the chelators to mobilise ^{59}Fe from ferritin in these studies may be via a direct or indirect mechanism.

In further studies, the ability of DFO (50 μM) and PCIH (50 μM) at inhibiting the uptake of ^{59}Fe from ^{59}Fe-Tf (0.75 μM) into ferritin by cardiomyocytes was assessed over an 18-h incubation (Fig. 2E and F). Both DFO and PCTH decreased the intensity of ^{59}Fe-ferritin bands to 2% and 50% of the control, respectively (Fig. 2E and F). Hence, the efficacy of DFO at preventing ^{59}Fe uptake into ferritin was greater than that found for PCTH in cardiomyocytes, which was in contrast to their similar efficacy at directly or indirectly mobilising ^{59}Fe from this molecule (Fig. 2C and D). Dialysis experiments [18] demonstrated that PCTH did not effectively remove ^{59}Fe from ^{59}Fe-Tf, being far less effective than DFO (Becker, E. and Richardson, D.R., data not shown). These studies suggested that the chelators were acting at a point distal to the high affinity Fe-binding sites of Tf.

Collectively, the results above from cardiomyocyte studies together with our previous investigations [20,21] prompted us to assess the Fe chelation efficacy of PCTH in vivo in mice using a well-established protocol [44].

3.2. The acute and subchronic effects of PCTH on ^{59}Fe excretion from mice

Administration of ^{59}Fe-lactoferrin to mice resulted in a marked increase in faecal ^{59}Fe excretion that peaked 8 h after the initial injection of the label (Fig. 3A). This protein was used to label mice with ^{59}Fe as it has been shown previously to result in hepatocyte Fe-loading [44]. After this initial increase, the excretion of ^{59}Fe returns to a low level, which was taken to be the baseline 28 h after the injection of the label (Fig. 3A). At this time, the first dose of chelator was administered. A second dose of the chelator was subsequently given 6 h later (Fig. 3A). A gradual increase in ^{59}Fe excretion occurred 4–6 h after the administration of the ligands (Fig. 3A). Over three experiments, administration of PCTH induced a significant ($P<0.01$) increase in ^{59}Fe excretion that continued for up to 20 h after the administration of two doses of treatment, after which it returned to the baseline level. This pattern of excretion was repeated when another dose of chelator was given (Fig. 3A). The animals remained healthy and no significant ($P>0.05$) weight change was observed even after a high dose of 200 mg/kg/*bd* of PCTH, PIH or L1 was administered over 2 days (Fig. 3B). The slight decrease in weights of the PIH- and L1-treated groups was not significant when compared to the control (Fig. 3B).

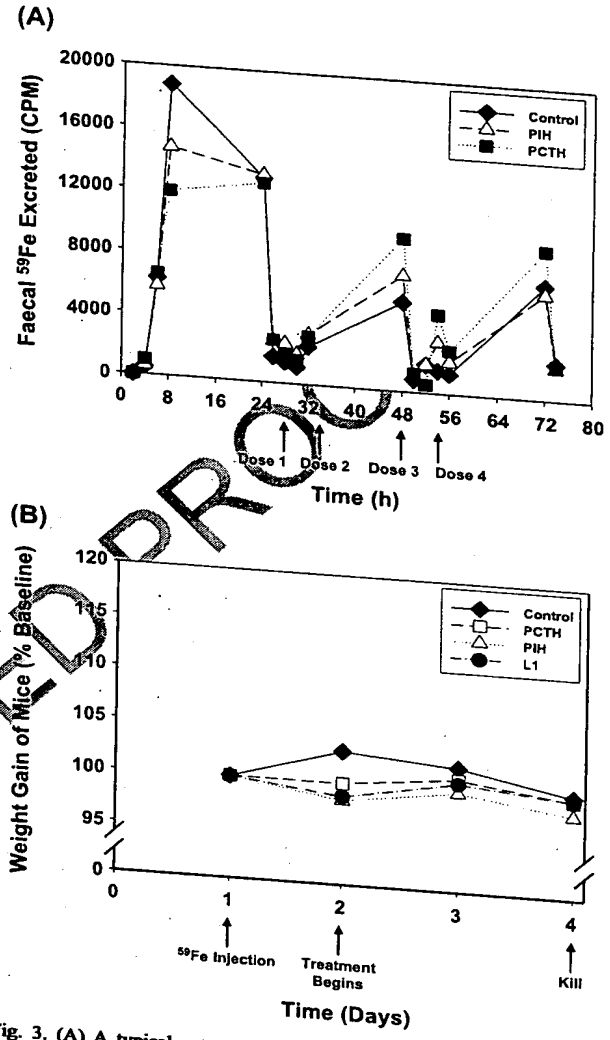


Fig. 3. (A) A typical pattern of faecal ^{59}Fe excretion in mice after oral administration of either vehicle (20% propylene glycol in 0.9% saline), PIH or PCTH. The baseline measurement of ^{59}Fe excretion was obtained 28 h after the ^{59}Fe-lactoferrin injection, upon which PIH (200 mg/kg/*bd*), PCTH (200 mg/kg/*bd*) or the vehicle control (20% propylene glycol in 0.9% saline) was administered over 2 days by gavage. This ^{59}Fe excretion profile was typical of three experiments performed with 3 mice per group per experiment. (B) Administration of chelators results in no weight change of mice over the same time course as shown in (A). The four treatment groups consisted of either the vehicle control (20% propylene glycol in 0.9% saline), PCTH (200 mg/kg/*bd*), PIH (200 mg/kg/*bd*), or L1 (200 mg/kg/*bd*), and administration occurred over 2 days by gavage. Data are expressed as the mean change in weight as compared to the initial weight. Results are means obtained from three experiments with 3 mice/group/experiment.

The main route of excretion for PCTH was via the faeces, as found for the parent compound, PIH [16–19,57,58]. Only a minute amount (<2%) of ^{59}Fe was excreted via the urinary route (data not shown). A dose-dependent increase in the faecal ^{59}Fe excretion was demonstrated in the PCTH-treated animals over the 2-day period, ranging from an increase of 14% compared to the vehicle control at a PCTH dose of 50

399 mg/kg/*bd* to 34% at a dose of 200 mg/kg/*bd* (Fig. 4A). The
400 increase in ^{59}Fe excretion at 200 mg/kg of PCTH was
401 comparable to its orally effective parent compound, PIH,
402 and also L1 at the same dose (Fig. 4A). The distribution of
403 intracellular ^{59}Fe after treatment with the vehicle control and
404 PCTH (50 and 75 mg/kg/*bd*) was assessed using native
405 PAGE ^{59}Fe-autoradiography on the livers from mice (Fig.

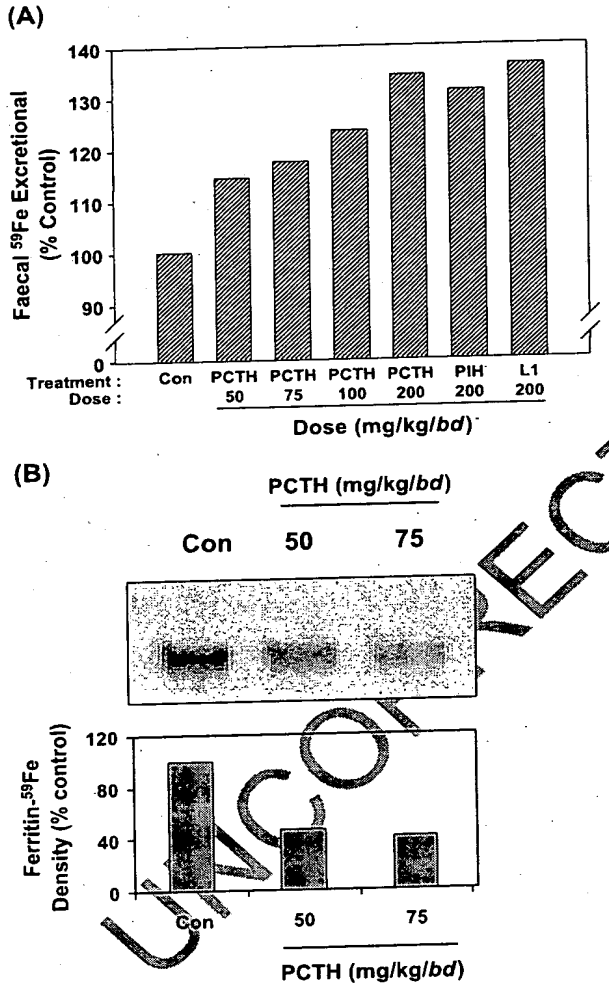


Fig. 4. (A) The dose response of orally administered PCTH on faecal ^{59}Fe excretion in mice compared to PIH and L1. Mice were administered by gavage for 2 days: PIH (200 mg /kg/*bd*), L1 (200 mg/kg/*bd*), or PCTH (50–200 mg/kg/*bd*). Results are expressed as a percentage of the vehicle control (i.e., 20% propylene glycol in 0.9% NaCl) and are the means of two experiments consisting of three mice per group per experiment. (B) Oral administration of PCTH to mice decreases ferritin-^{59}Fe levels as determined using native PAGE ^{59}Fe-autoradiography. Mice were labelled with ^{59}Fe-lactoferrin as described in the Materials and methods and then either the vehicle control or PCTH at doses of 50 or 75 mg/kg/*bd* were administered by gavage for 2 days. Livers were harvested at the end of the experiment. Lysates were then prepared and subjected to native PAGE ^{59}Fe-autoradiography (see Materials and methods for details). The results are typical from three separate experiments.

Table 1
Mean body weights of mice after 3 weeks of oral administration of PCTH (50–200 mg/kg/*bd*)

Parameter	Experimental groups			
	Group 1 (vehicle)	Group 2 (50 mg/kg/*bd*)	Group 3 (100 mg/kg/*bd*)	Group 4 (200 mg/kg/*bd*)
Day 1	21.70±1.08[a]	22.17±1.06	22.32±1.34	21.17±0.70
Day 8	22.24±1.24	22.04±1.46	22.35±1.82	18.17±14.16
Day 15	23.10±1.44	22.75±1.56	23.50±2.01	n/a
Day 21	23.69±1.47	23.53±1.68	23.70±2.34	n/a

[a] Results are expressed as the mean±S.D. and were obtained using 10 mice per group.

4B). Decreases of 55% and 60% in ferritin-^{59}Fe compared to the control were observed after administration of PCTH at 50 and 75 mg/kg/*bd*, respectively (Fig. 4B). In contrast to the data obtained from cardiomyocytes (Fig. 2C), ^{59}Fe ferritin in lysates from mouse liver ran as a single well-defined band, as seen for other cell types [43,45].

In subchronic studies, the vehicle control, PIH (75 mg/kg/*bd*), or PCTH (75 mg/kg/*bd*), was administered for 5 days/week for 3 weeks and faeces collected every 24 h. Similarly to the acute studies described above, PIH and PCTH at 75 mg/kg/*bd* increased faecal ^{59}Fe excretion to 140% and 145% of the vehicle control, respectively (data not shown). In addition, there was no change in the weight gain of mice after administration of the chelator for 3 weeks compared to that found for the vehicle control (data not shown).

3.3. Body weight and food intake after PCTH administration in mice

Considering the importance of determining toxicology in the early assessment of drugs for the treatment of human disease, we embarked upon studies to determine the effects of PCTH. Initially, toxicological assessment was examined by comparing oral PCTH administration (50, 100 and 200 mg/kg/*bd*) to the vehicle control over 21 days by examining mouse body weight and food intake. The body weights of the mice are shown in Table 1. Mice from all experimental groups except the high dose group of 200 mg/kg/*bd* exhibited normal weight gains over the 21-day period. Both the 50 and 100 mg/kg/*bd* groups gained a mean of 2% of their initial weight and the increments were not significantly different to the weight gain of the control group at 3%. Mice given 200 mg/kg/*bd* showed a reduction of 14.6% in body weight and signs of toxicity such as ungroomed coat and subdued appearance after 8 days of treatment (Table 1). These mice were subsequently sacrificed at day 8 for ethical reasons. Food intake by the mice was not significantly different across the treatment groups, with the exception of the 200 mg/kg/*bd* group, which consumed 40% less during the first week of treatment when compared to the control (data not shown).

Table 2
Haematological analysis of mice administered PCTH (50–200 mg/kg/*bd*) orally for 3 weeks

Parameter	Experimental Groups			
	Group 1 (Vehicle) Day 21	Group 2 (50 mg/kg/*bd*) Day 21	Group 3 (100 mg/kg/*bd*) Day 21	Group 4 (200 mg/kg/*bd*) Day 8
RBC ($\times 10^{12}$/L)	10.4±0.3[a]	10.6±0.3	10.4±0.5	9.9±0.5 (n=9)
Hb (g/L)	161±4	164±5	160±8	154±7 (n=9)
Hct (L/L)	0.47±0.01	0.47±0.01	0.46±0.02	0.44±0.02 (n=9)
MCV (fL)	45±1	45±1	44±1	45±1 (n=9)
MCH (pg)	15±1	15±1	15±0	16±1 (n=9)
MCHC (g/L)	346±6	348±5	350±5	347±3 (n=9)
WBC ($\times 10^9$/L)	2.4±1.1	2.6±1.0	2.8±1.7	3.3±1.3

Abbreviations: Hb, haemoglobin; Hct, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; WBC, white blood cells.

[a] Results are expressed as the mean±S.D. and were obtained from 10 mice per group unless indicated.

3.4. Haematology and serum biochemistry analyses after PCTH administration

The haematology and serum biochemistry analysis after administration of PCTH compared to the vehicle control are shown in Tables 2 and 3, respectively. No significant difference was observed in the various haematological parameters measured across all experimental groups (Table 2). The biochemistry analysis showed a slight elevation in the potassium levels and a slight reduction in the urea, ALT and AST levels in the PCTH-treated groups compared to the control (Table 3). However, these changes were not statistically significant.

3.5. Organ weights and histopathology after PCTH administration

Upon sacrifice, a gross pathological and histopathological assessment was conducted on all animals from control and treatment groups and no obvious abnormalities were observed. A dose-dependent increase in the weight of the liver was observed in the PCTH-treated groups as compared to the vehicle control (Table 4). However, this was not significant except at the highest dose of 200 mg/kg/*bd*. Histopathology of the liver of the PCTH-treated groups did not exhibit any atypical characteristics. Similarly, bone marrow smears of the PCTH-treated groups demonstrated no abnormalities when compared to the control group.

4. Discussion

One of the purposes of designing orally effective chelators was to overcome the numerous disadvantages of DFO, which include long subcutaneous infusions 12–24 h/day to effect significant Fe excretion [1–3]. In addition to L1, there are several chelators such as 4-[3,5-bis-(hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid (ICL670A) [50,51], 2-methyl-3-hydroxy-4H-benzopyran-4-one (MCOH) [52] and TREN-(Me-3,2-hydroxipyridonate) [53], which are under development and may be candidates for future oral treatment of Fe-overload disease [3]. The aroylhydra-

Table 3
Serum biochemistry analysis of mice after oral administration of PCTH (50–200 mg/kg/*bd*) for 3 weeks

Parameter	Experimental groups			
	Group 1 (vehicle), Day 21	Group 2 (50 mg/kg/*bd*), Day 21	Group 3 (100 mg/kg/*bd*), Day 21	Group 4 (200 mg/kg/*bd*), Day 8
Na (mmol/L)	137±9[a]	125±17	127±17 (n=9)	152±9 (n=6)
K (mmol/L)	4.8±0.7	5.3±0.9	5.2±1.5	6.0±0.9 (n=6)
Cl (mmol/L)	99±4	93±9	92±10	110±6 (n=6)
Na/K ratio	29.5±4.7	23.9±3.4	27.7±7.3	26.0±4.7 (n=6)
Urea (mmol/L)	7.9±1.9	7.0±0.9 (n=9)	7.1±2.6	7.1±1.5 (n=9)
P (mmol/L)	2.1±0.3 (n=8)	2.0±0.2 (n=5)	2.0±0.4	2.7±0.5 (n=9)
TP (g/L)	51±1 (n=4)	60*	55	[b]
ALB (g/L)	31±1 (n=6)	35±6 (n=3)	32±1	[b]
Glob (g/L)	21±1 (n=4)	25	22	[b]
Tbil (μmol/L)	10±2 (n=3)	11	11	[b]
AST (IU/L)	107±56 (n=6)	79	66±8	[b]
ALT (IU/L)	35±16 (n=7)	31±26 (n=5)	13±4	[b]

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cl, chloride; Glob, globulin; K, potassium; Na, sodium; Na/K, sodium/potassium ratio; P, phosphate; Tbil, total bilirubin; TP, total protein.

[a] Results are expressed as either the mean* from 2 mice or mean±S.D. from 10 mice unless indicated.

[b] Not performed.

t4.1 Table 4
t4.2 Organ weights of mice expressed as a percentage of body weight after oral administration of PCTH (50–200 mg/kg/*bd*) for 3 weeks

t4.3 Organs	Experimental Groups			
t4.4	Group 1 (Vehicle) Day 21	Group 2 (50 mg/kg/*bd*) Day 21	Group 3 (100 mg/kg/*bd*) Day 21	Group 4 (200 mg/kg/*bd*) Day 8
t4.5 Liver	4.77±0.38[a]	5.33±0.35	5.62±0.52	6.50±0.49[b]
t4.6 Kidney	1.49±0.09	1.50±0.13	1.49±0.12	1.60±0.12
t4.7 Gonads	0.80±0.05	0.78±0.14	0.71±0.15	0.76±0.10
t4.8 Spleen	0.29±0.02	0.28±0.03	0.28±0.04	0.25±0.06

t4.9 [a] Results are mean±S.D. of the organ weights and were obtained from 10 mice per group.
t4.10 [b] $P<0.05$ as compared to the control.

zone family of ligands [54–58], and in particular, PCTH, are also candidates [20,21].

Our present results demonstrated that PCTH was orally active in vivo in mice. Excretion of Fe was rapidly induced as shown by the 34% increase in faecal ^{59}Fe after an acute administration of PCTH (200 mg/kg/*bd*) over 2 days (Fig. 4A). More importantly, the increase in ^{59}Fe excretion in the PCTH-treated group was comparable to that induced by the well-known orally effective chelators, PIH and L1 [8,14,15,19,44] (Fig. 4A). In addition, our ^{59}Fe excretion data indicated that PCTH was rapidly absorbed across the gastrointestinal tract of mice (Fig. 3A). The oral effectiveness of PCTH was further substantiated by native PAGE ^{59}Fe-autoradiography studies which demonstrated a reduction in liver ferritin-^{59}Fe in mice administered PCTH (Fig. 4B). This was in accordance with our data in vitro showing that PCTH was capable of inducing ^{59}Fe release from cardiomyocytes (Fig. 2A) and also a number of other cell types [20,21]. Moreover, PCTH could inhibit ^{59}Fe uptake from ^{59}Fe-Tf into the ferritin of cardiomyocytes (Fig. 2E and F), again in accordance with previous studies using several cell lines [20].

Following the good efficacy shown by PCTH in acute studies in vivo, a toxicological assessment was conducted to assess the effects of daily PCTH administration over 21 days. These experiments demonstrated that PCTH was well tolerated at 100 mg/kg/*bd* when given orally to mice. Signs of toxicity were observed in the 200 mg/kg/*bd* group after 8 days of treatment. Therefore, for ethical reasons, animals in this latter treatment group were sacrificed at day 8 and all analyses for these mice were conducted at this time point. The biological functioning of the animal was assessed by haematological analysis and serum biochemistry. Mice in the PCTH-treated groups (50, 100 and 200 mg/kg *bd*) exhibited similar levels as the control group in all haematological and serum biochemistry parameters (Tables 2 and 3). Although the high dose PCTH group (200 mg/kg/*bd*) was unwell and showed signs of weight loss and ungroomed fur after 8 days of treatment, the haematological and biochemical indices demonstrated no significant change compared to the control (Tables 2 and 3).

The mechanism or site of toxicity of PCTH in the 200 mg/kg/*bd* group could not be determined in this study as no sign of abnormality was observed by organ histology. The weight of the liver as a percentage of body weight in the PCTH-treated groups showed a dose-dependent increase, which was not significant except at the highest dose (Table 4). Further studies are required to investigate the cause and mechanism of PCTH-induced toxicity in mice. Considering this, it must be noted that the animals used were not Fe-loaded and the effect observed could be due to Fe-depletion and dysfunction of vital Fe-containing metabolic pathways, such as DNA synthesis [59, 60]. However, this suggestion is not in accordance with the lack of alteration in haematological parameters such as red blood cell (RBC) number or haemoglobin (Hb) concentration (Table 2), which are indices of Fe status. Alternatively, the Fe complex of the ligand could possess cytotoxic properties by inducing the formation of reactive oxygen species for instance [60]. It is of interest that in cell culture studies, PCTH showed low anti-proliferative activity which was less than that observed with DFO [20]. The PCTH chelator also did not markedly inhibit cellular ^{3}H-thymidine incorporation, being far less effective than the cytotoxic ligand, 311 [20]. Furthermore, studies in vitro examining the redox activity of the PCTH–Fe complex did not show any pronounced activity nor did this chelator avidly bind to DNA or induce its degradation in intact cells [23].

The design of PCTH was based on PIH and its analogues which have been shown in a range of investigations to be orally effective chelators in vivo in animals as well as in human trials [14–22,55–58]. Our previous studies have shown that the PCIH class of compounds are known to bind Fe(II) and Fe(III) effectively [33,34]. Indeed, this could be partly responsible for their high activity, as cellular Fe is known to exist in both the Fe(II) and Fe(III) states [59–61].

In summary, PCTH has demonstrated good oral efficacy and a rapid mode of action in terms of inducing Fe excretion from mice. The effect exerted by PCTH was equivalent to the known orally active ligands, PIH and L1, indicating its potential as a substitute for DFO. Moreover, the chelator was shown to be well tolerated at doses of 100 mg/kg/*bd* over 3 weeks. These promising results warrant the further investigation of PCTH in vivo as a prospective therapeutic agent for Fe-loading diseases such as β-thalassaemia major and FA.

Acknowledgements

Children's Cancer Institute Australia for Medical Research is affiliated with the University of New South Wales and Sydney Children's Hospital. This project was supported by grants from Metabolic Pharmaceuticals Ltd. (Melbourne, Australia), a fellowship and project grants from the National Health and Medical Research Council and Discovery grants from the Australian Research Council (ARC). The authors sincerely thank Dr. Ralph Watts for his assistance with preparing the graphs and tables. Dr. Neil Davies, Ms. Louise Dunn and Mr. Nghia Le are kindly acknowledged for their critical examination of the manuscript prior to submission. Finally, the authors acknowledge ICP Firefly Pty Ltd, Sydney, for technical assistance in the toxicological study using mice.

References

[1] C. Hershko, Control of disease by selective iron depletion: a novel therapeutic strategy utilizing iron chelators, Bailliére's, Clin. Haematol. 7 (1994) 965–1000.
[2] N.F. Olivieri, G.M. Brittenham, Iron chelation therapy and the treatment of thalassemia, Blood 89 (1997) 739–761.
[3] C. Wong, D.R. Richardson, Beta-thalassaemia: emergence of new and improved iron chelators for treatment, Int. J. Biochem. Cell Biol. 35 (2003) 1144–1149.
[4] D.R. Richardson, The controversial role of deferiprone in the treatment of thalassemia, J. Lab. Clin. Med. 137 (2001) 324–329.
[5] L.J. Anderson, B. Wonke, E. Prescott, S. Holden, J.M. Walker, D.J. Pennell, Comparison of effects of oral deferiprone and subcutaneous desferrioxamine on myocardial iron concentrations and ventricular function in beta-thalassaemia, Lancet 360 (2002) 516–520.
[6] A. Maggio, G. D'Amico, A. Morabito, M. Capra, C. Ciaccio, P. Cianciulli, F. Di Gregorio, G. Garozzo, R. Malizia, C. Magnano, A. Mangiagli, G. Quarta, M. Rizzo, D.G. D'Ascola, A. Rizzo, A. Midiri, Deferiprone versus deferoxamine in patients with thalassemia major: a randomized clinical trial, Blood Cells Mol. Diseases 28 (2002) 196–208.
[7] A.R. Cohen, R. Galanello, A. Piga, V. De Sanctis, F. Tricta, Safety and effectiveness of long-term therapy with the oral iron chelator deferiprone, Blood 102 (2003) 1583–1587.
[8] G.J. Kontoghiorghes, K. Neocleous, A. Kolnagou, Benefits and risks of deferiprone in iron overload in thalassaemia and other conditions: comparison of epidemiological and therapeutic aspects with deferoxamine, Drug Safety 26 (2003) 553–584.
[9] N.F. Olivieri, G.M. Brittenham, C.E. McLaren, D.M. Templeton, R.G. Cameron, R.A. McClelland, A.D. Burt, K.A. Fleming, Long-term safety and effectiveness of iron-chelation therapy with deferiprone for thalassemia major, N. Engl. J. Med. 339 (1998) 417–423.
[10] A.V. Hoffbrand, F. Al-Refaie, B. Davis, N. Siritanakatkul, B.F. Jackson, J. Cochrane, E. Prescott, B. Wonke, Long-term trial of deferiprone in 51 transfusion-dependent iron overloaded patients, Blood 91 (1998) 295–300.
[11] C. Hershko, M.D. Cappellini, R. Galanello, A. Piga, G. Tognoni, G. Masera, Purging iron from the heart, Br. J. Haematol. 125 (2004) 545–551.
[12] K.H. Wu, J.S. Chang, C.H. Tsai, C.T. Peng, Combined therapy with deferiprone and desferrioxamine successfully regresses severe heart failure in patients with beta-thalassemia major, Ann. Hematol. 83 (2004) 471–473.
[13] S. Gomber, R. Saxena, N. Madan, Comparative efficacy of desferrioxamine, deferiprone and in combination on iron chelation in thalassemic children, Indian Pediatr. 41 (2004) 21–27.
[14] P. Ponka, J. Borova, J. Neuwirt, O. Fuchs, Mobilization of iron from reticulocytes. Identification of pyridoxal isonicotinoyl hydrazone as a new iron chelating agent, FEBS Lett. 97 (1979) 317–321.
[15] P. Ponka, J. Borova, J. Neuwirt, O. Fuchs, E. Necas, A study of intracellular iron metabolism using pyridoxal isonicotinoyl hydrazone and other synthetic chelating agents, Biochim. Biophys. Acta 586 (1979) 278–297.
[16] T. Hoy, J. Humphreys, A. Jacobs, A. Williams, P. Ponka, Effective iron chelation following oral administration of an isoniazid pyridoxal hydrazone, Br. J. Haematol. 43 (1979) 443–449.
[17] M. Cikrt, P. Ponka, E. Necas, J. Neuwirt, Biliary iron excretion in rats following pyridoxal isonicotinoyl hydrazone, Br. J. Haematol. 45 (1980) 275–283.
[18] E. Baker, D. Richardson, S. Gross, P. Ponka, Evaluation of the iron chelation potential of hydrazones of pyridoxal, salicylaldehyde and 2-hydroxy-1-naphthylaldehyde using the hepatocyte in culture, Hepatology 15 (1992) 492–501.
[19] G.M. Brittenham, Pyridoxal isonicotinoyl hydrazone: an effective chelator after oral administration, Semin. Hematol. 27 (1990) 112–116.
[20] E. Becker, D.R. Richardson, Development of novel aroylhydrazone ligands for iron chelation therapy: 2-pyridylcarboxaldehyde isonicotinoyl hydrazone analogs, J. Lab. Clin. Med. 134 (1999) 510–521.
[21] D.R. Richardson, C. Mouralian, P. Ponka, E. Becker, Development of potential iron chelators for the treatment of Friedreich's ataxia: ligands that mobilize mitochondrial iron, Biochim. Biophys. Acta 1536 (2001) 133–140.
[22] D.R. Richardson, P.V. Bernhardt, E. Becker, Iron Chelators and Uses Thereof, International Patent Application No. PCT/AU00/01050. 2000 4th September, 2000.
[23] T.B. Chaston, D.R. Richardson, Interactions of the pyridine-2-carboxaldehyde isonicotinoyl hydrazone class of chelators with iron and DNA: implications for toxicity in the treatment of iron overload disease, J. Biol. Inorg. Chem. 8 (2003) 427–438.
[24] M.B. Delatyki, J. Camakaris, H. Brooks, T. Evans-Whipp, D.R. Thorburn, R. Williamson, S.M. Forrest, Direct evidence that mitochondrial iron accumulation occurs in Friedreich ataxia, Ann. Neurol. 45 (1999) 673–675.
[25] H. Puccio, D. Simon, M. Cossee, P. Criqui-Filipe, F. Tiziano, J. Melki, C. Hindelang, R. Matyas, P. Rustin, M. Koenig, Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe–S enzyme deficiency followed by intramitochondrial iron deposits, Nat. Genet. 27 (2001) 181–186.
[26] E. Becker, D.R. Richardson, Frataxin: its role in iron metabolism and the pathogenesis of Friedreich's ataxia, Int. J. Biochem. Cell Biol. 33 (2001) 1–10.
[27] M. Pandolfo, Friedreich ataxia, Semin. Pediatr. Neurol. 10 (2003) 163–172.
[28] E. Becker, J. Greer, P. Ponka, D.R. Richardson, Erythroid differentiation and protoporphyrin IX down-regulate frataxin expression in Friend cells: characterisation of frataxin expression compared to molecules involved in iron metabolism and hemoglobinisation, Blood 99 (2002) 3813–3822.
[29] T. Yoon, J.A. Cowan, Frataxin-mediated iron delivery to ferrochelatase in the final step of heme biosynthesis, J. Biol. Chem. 279 (2004) 25943–25946.
[30] G. Isaya, H.A. O'Neill, O. Gakh, S. Park, R. Mantcheva, S.M. Mooney, Functional studies of frataxin, Acta Paediatr., Suppl. 93 (2004) 68–71.
[31] A. Wong, J. Yang, P. Cavadini, C. Gellera, B. Lonnerdal, F. Taroni, G. Cortopassi, The Friedreich's ataxia mutation confers cellular sensitivity to oxidant stress which is rescued by chelators of iron and calcium and inhibitors of apoptosis, Hum. Mol. Genet. 8 (1999) 425–430.

[32] J.L. Bradely, S. Homayoun, P.E. Hart, A.H. Shapira, J.M. Cooper, Role of oxidative damage in Friedreich's ataxia, Neurochem. Res. 29 (2004) 561–567.
[33] P.V. Bernhardt, P. Chin, D.R. Richardson, Unprecedented oxidation of a biologically active aroylhydrazone chelator catalysed by iron(III): serendipitous identification of diacylhydrazone ligands with high iron chelation efficacy, J. Biol. Inorg. Chem. 6 (2001) 801–809.
[34] C.M. Armstrong, P.V. Bernhardt, P. Chin, D.R. Richardson, Structural variations and formation constants of first row transition metal complexes of biologically active aroylhydrazones, Eur. J. Inorg. Chem. (2003) 1145–1156.
[35] G. Link, A. Pinson, C. Hershko, Heart cells in culture: a model of myocardial iron overload and chelation, J. Lab. Clin. Med. 106 (1985) 147–153.
[36] C. Hershko, G. Link, M. Tzahor, J.P. Kaltwasser, P. Athias, A. Grynberg, A. Pinson, Anthracycline toxicity is potentiated by iron and inhibited by deferoxamine: studies in rat heart cells in culture, J. Lab. Clin. Med. 122 (1993) 245–251.
[37] G. Link, R. Tirosh, A. Pinson, C. Hershko, Role of iron in the potentiation of anthracycline cardiotoxicity: identification of heart cell mitochondria as a major site of iron anthracycline interaction, J. Lab. Clin. Med. 127 (1996) 272–278.
[38] A. Terman, U.T. Brunk, On the degradability and exocytosis of ceroid/lipofuscin in cultured rat cardiac myocytes, Mech. Ageing Dev. 100 (1998) 145–156.
[39] E.J. Goncharova, Z. Kam, B. Geiger, The involvement of adherens juction components in myofibrillogenesis in cultured cardiac myocytes, Development 114 (1992) 173–183.
[40] J.C. Kwok, D.R. Richardson, Anthracyclines induce accumulation of ferritin iron in normal and neoplastic cells: inhibition of the ferritin Fe mobilization pathway, Mol. Pharmacol. 63 (2003) 849–861.
[41] D.R. Richardson, E. Baker, The uptake of iron and transferrin by the human malignant melanoma cell, Biochim. Biophys. Acta 1053 (1990) 1–12.
[42] D.R. Richardson, E. Baker, Two mechanisms of iron uptake from transferrin by melanoma cells. The effect of desferrioxamine and ferric ammonium citrate, J. Biol. Chem. 267 (1992) 13972–13979.
[43] D.R. Richardson, K. Milnes, The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents: II. The mechanism of action of ligands derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone, Blood 89 (1997) 3025–3038.
[44] J.B. Porter, J. Morgan, K.P. Hoyes, J.C. Burke, E.R. Huehns, R.C. Hider, Relative oral efficacy and acute toxicity of hydroxypyridin-4-one iron chelators in mice, Blood 76 (1990) 2389–2396.
[45] D.R. Richardson, P. Ponka, D. Vyoral, Distribution of iron in reticulocytes after inhibition of heme synthesis with succinylacetone: examination of the intermediates involved in iron metabolism, Blood 87 (1996) 3477–3488.
[46] D.R. Richardson, E.H. Tran, P. Ponka, The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents, Blood 86 (1995) 4295–4306.
[47] G. Darnell, D.R. Richardson, The potential of analogues of the pyridoxal isonicotinoyl hydrazone class as effective anti-proliferative agents III: the effect of the ligands on molecular targets involved in proliferation, Blood 94 (1999) 781–792.
[48] C.H. Campbell, D. Crocker, J.J. Cruntmeir, M. Head, T. Kelly, M.I. Langfur, A.H. Leimer, Purification of a novel glycosylated ferritin from horse heart, J. Cell. Biochem. 53 (1993) 420–432.
[49] S.C. Andrews, A. Treffry, P.M. Harrison, A new form of ferritin heterogeneity explained. Isolation and identification of a nineteen-amino-acid-residue fragment from siderosomal ferritin of rat liver, Biochem. J. 245 (1987) 447–453.
[50] C. Hershko, A.M. Konijn, H.P. Nick, W. Breuer, Z.I. Cabantchik, G. Link, ICL670A: a new synthetic oral chelator: evaluation in hypertransfused rats with selective radioiron probes of hepatocellular and reticuloendothelial iron stores and in iron-loaded rat heart cells in culture, Blood 97 (2001) 1115–1122.
[51] R. Galanello, A. Piga, D. Alberti, M.C. Rouan, H. Bigler, R. Sechaud, Safety tolerability, and pharmacokinetics of ICL670, a new orally active iron-chelating agent in patients with transfusion-dependent iron overload due to beta-thalassemia, J. Clin. Pharmacol. 43 (2003) 565–572.
[52] M. Ferrali, D. Donati, S. Bambagioni, M. Fontani, G. Giorgi, A. Pietrangelo, 3-Hydroxy (4*H*)-benzopyran-4-ones as potential iron chelating agents in vivo, Bioorg. Med. Chem. 9 (2001) 3041–3047.
[53] R.A. Yokel, A.M. Fredenburg, P.W. Durbin, J. Xu, M.K. Rayens, K.N. Raymond, The hexadentate hydroxypyridinonate TREN-(Me-3,2-HOPO) is a more orally active iron chelator than its bidentate analogue, J. Pharm. Sci. 89 (2000) 545–555.
[54] P. Ponka, D. Richardson, E. Baker, H.M. Schulman, J.T. Edward, Effect of pyridoxal isonicotinoyl hydrazone (PIH) and other hydrazones on iron release from macrophages, reticulocytes and hepatocytes, Biochim. Biophys. Acta 967 (1988) 122–129.
[55] D.R. Richardson, P. Ponka, Pyridoxal isonicotinoyl hydrazone and its analogs: potential orally effective iron-chelating agents for the treatment of iron overload disease, J. Lab. Clin. Med. 131 (1998) 306–315.
[56] K. Blaha, M. Cikrt, J. Nerudova, P. Ponka, Biliary iron excretion in rats following treatment with analogs of pyridoxal isonicotinoyl hydrazone, Blood 91 (1998) 4368–4372.
[57] C. Hershko, S. Avramovici-Grisaru, G. Link, L. Gelfand, S. Sarel, Mechanism of in vivo iron chelation by pyridoxal isonicotinoyl hydrazone and other imino derivatives of pyridoxal, J. Lab. Clin. Med. 98 (1981) 99–108.
[58] G. Link, P. Ponka, A.M. Konijn, W. Breuer, Z.I. Cabantchik, C. Hershko, Effects of combined chelation treatment with pyridoxal isonicotinoyl hydrazone analogs and deferoxamine in hypertransfused rats and in iron-loaded rat heart cells, Blood 101 (2003) 4172–4179.
[59] D.R. Richardson, P. Ponka, The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells, Biochim. Biophys. Acta 1331 (1997) 1–40.
[60] T.B. Chaston, D.R. Richardson, Iron chelators for the treatment of iron overload disease: the relationship between structure, redox activity and toxicity, Am. J. Hematol. 73 (2003) 200–210.
[61] T.G. St. Pierre, D.R. Richardson, E. Baker, J. Webb, A low-spin iron complex in human melanoma and rat hepatoma cells and a high-spin iron(II) complex in rat hepatoma cells, Biochim. Biophys. Acta 1135 (1992) 154–158.

About Thalassemia

CAF Home
About Thalassemia
Medical Information
Local Chapters
Legislative Advocacy
How You Can Help
Online Donation Form

TAG
TAG 2004 Conference
About Us/ Contact Us
Medical Fellowships
News Archives
Links & Resources

Login

Thalassemia Info | Finding a Cure | Thalassemia Trait | CAF on Dialogue 1

What is Thalassemia? FAQ

What Is Thalassemia?

To download a pdf file with information on **alpha thalassemia**, please click here.

To download a pdf file with information on **beta thalassemia**, please click here.

A Basic Description

Thalassemia is the name of a group of genetic blood disorders. To understand how thalassemia affects the human body, you must first understand a little about how blood is made.

Hemoglobin is the oxygen-carrying component of the red blood cells. It consists of two different proteins, an **alpha** and a **beta**. If the body doesn't produce enough of either of these two proteins, the red blood cells do not form properly and cannot carry sufficient oxygen. The result is anemia that begins in early childhood and lasts throughout life.

Since thalassemia is not a single disorder but a group of related disorders that affect the human body in similar ways, it is important to understand the differences between the various types of thalassemia.

Alpha Thalassemia

To download a pdf file with information on **alpha thalassemia**, please click here.

People whose hemoglobin does not produce enough alpha protein have **alpha thalassemia**. It is commonly found in Africa, the Middle East, India, Southeast Asia, souther China, and occasionally the Mediterranean region.

There are four types of alpha thalassemia that range from mild t severe in their effect on the body.

Silent Carrier State. This condition generally causes no health problems because the lack of alpha protein is so small that the hemoglobin functions normally. It is called "silent carrier" because of how difficult it is to detect. Silent carrier state is "diagnosed" by deduction when an apparently normal individua has a child with hemoglobin H disease or alpha thalassemia trai

Hemoglobin Constant Spring. This is an unusual form of Silent Carrier state that is caused by a mutation of the alpha globin. It called Constant Spring after the region of Jamaica in which it w discovered. As in silent carrier state, an individual with this condition usually experiences no related health problems.

Alpha Thalassemia Trait or *Mild Alpha Thalassemia*. In this condition, the lack of alpha protein is somewhat greater. Patient with this condition have smaller red blood cells and a mild anemia, although many patients do not experience symptoms. However, physicians often mistake mild alpha thalassemia for iron deficiency anemia and prescribe iron supplements that have no effect on the anemia.

Hemoglobin H Disease. In this condition, the lack of alpha protein is great enough to cause severe anemia and serious healt problems such as an enlarged spleen, bone deformities and fatigue. It is named for the abnormal hemoglobin H (created by the remaining beta globin) that destroys red blood cells.

Hemoglobin H-Constant Spring. This condition is more severe than hemoglobin H disease. Individuals with this condition tend to have a more severe anemia and suffer more frequently from enlargement of the spleen and viral infections.

Homozygous Constant Spring. This condition is a variation of hemoglobin H-Constant Spring that occurs when two Constant Spring carriers pass their genes on to their child (as opposed to hemoglobin H Constant Spring, in which one parent is a Consta Spring Carrier and the other a carrier of alpha thalassemia trait) This condition is generally less severe than hemoglobin H Constant Spring and more similar to hemoglobin H disease.

Hydrops Fetalis or *Alpha Thalassemia Major*. In this condition, there are no alpha genes in the individual's DNA, which causes the gamma globins produced by the fetus to form an abnormal hemoglobin called hemoglobin Barts. Most individuals with thi condition die before or shortly after birth. In some extremely ra cases where the condition is discovered before birth, in utero blood transfusions have allowed the birth of children with hydrops fetalis who then require lifelong blood transfusions and medical care.

Beta Thalassemia

People whose hemoglobin does not

To download a pdf file with information on **beta thalassemia**, please click

produce enough beta protein have **beta thalassemia**. It is found in people of Mediterranean descent, such as Italians and Greeks, and is also found in the Arabian Peninsula, Iran, Africa, Southeast Asia and southern China. here.

There are three types of beta thalassemia that also range from mild to severe in their effect on the body.

Thalassemia Minor or *Thalassemia Trait*. In this condition, the lack of beta protein is not great enough to cause problems in the normal functioning of the hemoglobin. A person with this condition simply carries the genetic **trait** for thalassemia and w usually experience no health problems other than a possible mil anemia. As in mild alpha thalassemia, physicians often mistake the small red blood cells of the person with beta thalassemia minor as a sign of iron-deficiency anemia and incorrectly prescribe iron supplements.

Thalassemia Intermedia. In this condition the lack of beta prote in the hemoglobin is great enough to cause a moderately severe anemia and significant health problems, including bone deformities and enlargement of the spleen. However, there is a wide range in the clinical severity of this condition, and the borderline between thalassemia intermedia and the most severe form, thalassemia major, can be confusing. The deciding factor seems to be the amount of blood transfusions required by the patient. The more dependent the patient is on blood transfusions the more likely he or she is to be classified as thalassemia major Generally speaking, patients with thalassemia intermedia need blood transfusions to improve their quality of life, but not in order to survive.

Thalassemia Major or *Cooley's Anemia*. This is the most severe form of beta thalassemia in which the complete lack of beta protein in the hemoglobin causes a life-threatening anemia that requires regular blood transfusions and extensive ongoing medical care. These extensive, lifelong blood transfusions lead iron-overload which must be treated with chelation therapy to prevent early death from organ failure.

Other Forms of Thalassemia

In addition to the alpha and beta thalassemias, there are other related disorders that occur when the gene for alpha or beta thalassemia combines with an abnormal or mutant gene.

E Beta Thalassemia. Hemoglobin E is one of the most common abnormal hemoglobins. It is usually found in people of Southea Asian ancestry, such as Cambodians, Vietnamese and Thai. When combined with beta thalassemia, hemoglobin E produces beta thalassemia, a moderately severe anemia which is similar i symptoms to beta thalassemia intermedia.

Sickle Beta Thalassemia. This condition is caused by a combination of beta thalassemia and hemoglobin S, the abnorm hemoglobin found in people with sickle cell disease. It is commonly found in people of Mediterranean ancestry, such as Italians, Greeks and Turks. The condition varies according to th amount of normal beta globin produced by the beta gene. When no beta globin is produced by the beta gene, the condition is almost identical with sickle cell disease. The more beta globin produced by the beta gene, the less severe the condition.

Treatment of Thalassemia

Blood Transfusions

The most common treatment for all major forms of thalassemia is **red blood cell transfusions**. These transfusions are necessary to provide the patient with a temporary supply of healthy red blood cells with normal hemoglobin capable of carrying the oxygen that the patient's body needs.

While thalassemia patients were given infrequent transfusions in the past, clinical research led to a more frequent program of regular blood cell transfusions that has greatly improved the patients' quality of life. Today, most patients with a major form thalassemia receive red blood cell transfusions every two to thre weeks, amounting to as much as 52 pints of blood a year.

Iron Overload

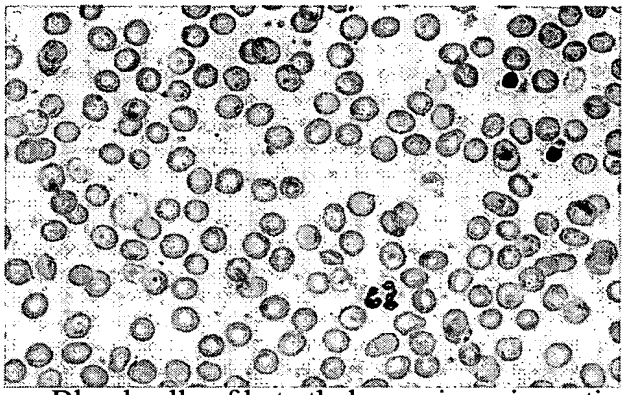
Because there is no natural way for the body to eliminate iron, t iron in the transfused blood cells builds up in a condition known as "iron overload" and becomes toxic to tissues and organs, particularly the liver and heart. Iron overload typically results in the patient's early death from organ failure.

Chelation Therapy

To help remove excess iron, patients undergo the difficult and painful infusion of a drug, **Desferal**. A needle is attached to a small battery-operated infusion pump and worn under the skin o the stomach or legs five to seven times a week for up to twelve hours. Desferal binds iron in a process called "chelation." Chelated iron is later eliminated, reducing the amount of stored iron.

The Compliance Problem

Compliance with Desferal is vital to the thalassemia patient's lo term survival. However, many patients find the treatment so difficult that they do not keep up with it or abandon treatment altogether. Lack of compliance with chelation therapy leads to accelerated health problems and early death. To combat the compliance problem, researchers are at work on less stressful new chelators that can improve patient compliance.



Blood cells of beta thalassemia major patient

Cooley's Anemia Foundation, Inc. TEL: 800 522-7222 FAX: 718 321-3340 info@cooleysanemia.org